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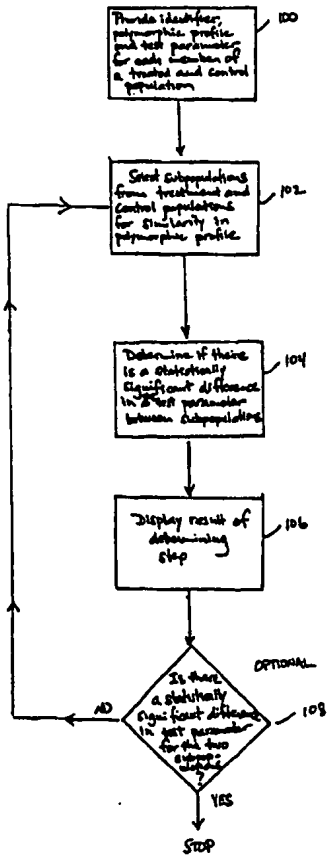
(54) Title: METHODS TO REDUCE VARIANCE IN TREATMENT STUDIES USING GENOTYPING

(57) Abstract

The present invention provides methods, computer programs and computerized systems useful for evaluating the efficacy of various types of treatment procedures (e.g., clinical trials) as a function of the genotype of a subject. By matching treatment and control groups genetically, the methods and systems of the invention reduce the total variance of the study, thereby allowing trials examining the efficacy or effect of treatment procedures to be conducted with fewer subjects, with increased confidence values, and/or with increased precision or discriminatory power. Certain methods of the invention involve selecting treated and control subpopulations of subjects from treated and control populations for similarity in polymorphic profile, wherein the treated and control populations have been treated with a treatment and control procedure, respectively. A determination is then made whether there is a statistically significant difference in a test parameter between the treated and control subpopulations as an assessment of the test procedure.



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METHODS TO REDUCE VARIANCE IN TREATMENT STUDIES USING GENOTYPING

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/110,668, filed December 2, 1998, which is incorporated by reference in its entirety for all purposes.

FIELD OF INVENTION

The present invention resides in the fields of medicine, genetics and statistics.

BACKGROUND OF THE INVENTION

The conduct and design of studies for investigating treatment efficacy such as clinical trials aims to eliminate the bias that can arise from "random" biological influence be they genetic or environmental, as well as bias introduced by the investigator wittingly or otherwise. One approach for reducing bias is to randomize individuals to either treatment or control groups with the view that if the individuals in the two groups are unrelated genetically and live independent of one another, then both genetic and environmental influences on the trial will be balanced in the two arms of the study. An immediate consequence of randomization in this way, however, is that the variance of the biological condition measured is greater than if each case is matched for genetic and known environmental influences.

One method for determining genetic variability is by assessment of polymorphic profile. Polymorphisms refer to the coexistence of multiple forms of a sequence in a population. Several different types of polymorphisms have been reported. A

restriction fragment length polymorphism (RFLP), for example, means a variation in DNA sequence that alters the length of a restriction fragment (see, *e.g.*, Botstein et al., Am. J. Hum. Genet. 32:314-331 (1980)). Short tandem repeats (STRs), as the name implies, are short tandem repeats that consist of tandem di-, tri- and tetra-nucleotide repeat motifs. Such polymorphisms are also sometimes referred to as variable number tandem repeat (VNTR) polymorphisms (see, *e.g.*, U.S. Patent No. 5,075,217; Armour et al., FEBS Lett. 307:113-115 (1992); and Horn et al., WO 91/14003).

By far the most common form of polymorphisms are those involving single nucleotide variations between individuals of the same species; such polymorphisms are called single nucleotide polymorphisms, or simply SNPs. Some SNPs that occur in protein coding regions give rise to the expression of variant or defective proteins, and thus are potentially the cause of a genetic disease. Even SNPs that occur in non-coding regions can nonetheless result in defective protein expression (*e.g.*, by causing defective splicing). Other SNPs have no phenotypic effects.

SUMMARY OF THE INVENTION

Certain methods of the invention are designed to provide an assessment of the efficacy of a treatment procedure. In general, such methods involve selecting treated and control subpopulations from treated and control populations of subjects, wherein the treated population has been treated with a treatment procedure and the control population has been treated with a control procedure. The subjects in both the treated and control populations have been characterized for polymorphic profile and are selected because they have similar polymorphic profiles. A determination is then made whether there is a statistically significant difference in a test parameter between the treated and control subpopulations. In general, such a statistically significant difference indicates that there is a correlation between the type of treatment and one or more polymorphic forms within the polymorphic profile for which the treated and control subpopulations were selected.

In some instances, especially when a significant difference is not found, the selecting and determining steps are repeated one or more times. In such additional

cycles, the polymorphic profile for which the treated and control groups are selected differs from the polymorphic profile selected for in previous cycles. The polymorphic profile for which the subpopulations are selected can vary in terms of numbers of polymorphic forms within the profile and the extent of similarity in profiles between treated and control groups. For example, the polymorphic profile can include a single polymorphic form, but more typically includes a plurality of polymorphic forms (e.g., 10 or up to 100 polymorphic forms or more). In most instances, the polymorphic profiles of the subpopulations have at least 10%, 50%, 75% or up to 100% identity.

Certain methods of the invention are directed towards methods for performing clinical trials. Some of these methods initially involve treating a treated population and a control population of patients having the same disease with a drug and a control procedure (e.g., treating with a placebo or with a different amount of the drug or according to a different treatment schedule), respectively. A subpopulation of patients is then selected from each of the treated and control populations for similarity in a polymorphic profile. A determination is then made whether treatment with the drug correlates with status of the disease in the subpopulations to assess the efficacy of the drug in treating the disease. With these methods too, a correlation indicates that at least one or more polymorphic forms within the polymorphic profiles correlates with treatment efficacy.

Some methods of the invention are computerized methods. For example, certain methods of the invention include providing a database capable of storing: (1) designations for each member of a treated population treated according to a treatment procedure and designations for each member of a control population treated according to a control procedure, (2) designations for a polymorphic profile for each member of the treated and control populations, and (3) designations for a test parameter for each member of the treated and control populations. Using the database, subpopulations from each of the treated and control populations are selected for similarity in polymorphic profile. A determination is then made to ascertain whether there is a statistically significant difference in the test parameter between the subpopulations. The output from the determining step is then displayed on an output device (e.g., a monitor or video

display).

In another aspect, the invention provides various computer systems and programs. For instance, certain computer products for assessing a treatment procedure are provided. Some systems include program products that generally include code for providing or receiving data, wherein the data includes: (1) designations for each member of a treated population treated according to a treatment procedure and for each member of a control population treated according to a control procedure, (2) designations for a polymorphic profile for each member of the treated and control populations, and (3) designations for a test parameter for each member of the treated and control populations. The program also includes code for selecting a subpopulation from each of the treatment and control populations that have a similar polymorphic profile, code for determining whether there is a statistically significant difference in the test parameter between the subpopulations and code for displaying an output that indicates whether a statistically significant difference was found between the subpopulations. The code is typically stored on a computer readable storage medium.

The invention further provides a computerized system for assessing treatment procedures. Some systems generally include a memory, a system bus and a processor. The processor is operatively disposed to provide or receive data, wherein the data includes: (1) designations for each member of a treated population having been treated according to a treatment procedure and for each member of a control population treated according to a control procedure, (2) designations for a polymorphic profile for each member of the treated and control populations, and (3) designations for a test parameter for each member of the treated and control populations. The processor is further disposed to select a subpopulation from each of the treatment and control populations that have a similar polymorphic profile and determine whether there is a statistically significant difference in the test parameter between the subpopulations. The microprocessor is also capable of displaying an output indicating whether a statistically significant difference was found between the subpopulations.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1 and 2 depict computer systems for implementing the methods of the invention.

FIG. 3 is a flow chart for a method of assessing a treatment procedure according to the present invention.

DETAILED DESCRIPTION

I. Definitions

A "treatment procedure" refers to methods or processes that are performed on a member of a treated or treatment population. In general the treatment procedure is a process performed on a subject to affect some biological condition, susceptibility, or resistance of the subject. Examples of treatment procedures include, but are not limited to, treatment with pharmaceutical compounds or other biologics (including, for example, recombinantly produced proteins), surgical procedures and various behavioral therapies (*e.g.*, prescribed diet and/or exercise regimes). Treatment procedures can be prophylactic or therapeutic. For example, a treatment procedure can include treating members of a treatment population with a vaccine.

In like manner, a "control procedure" refers to methods that are performed on a member of a control population. The control procedure can differ from the treatment procedure in quantitative or qualitative aspects. For example, the members of a treatment population can receive a pharmaceutical composition, whereas the control population receives a placebo (*i.e.*, no pharmaceutical composition). In other instances, the control procedure involves administration of a drug at different concentrations than the treatment procedure or can involve a different schedule for administering the pharmaceutical composition relative to the treatment procedure.

A "clinical study" is an inquiry into the cause and sometimes treatment of a particular phenotype that is represented by at least one random variable. This phenotype may often be a disease state or a measure of disease severity. A clinical study can take

the form of a case-control study (for a discrete random variable, the groups being affected and unaffected individuals) or a single population study where the cause of the degree or severity of the phenotype is being investigated (for example, a quantitative study can examine blood pressure, blood glucose, etc.).

A "treatment study" is an inquiry into the effect or influence a particular treatment procedure has on a biological condition, biological susceptibility or biological resistance of a subject. The study can be quite structured, formal and extensive in scope, or can be relatively unstructured and of limited scope. For example, a treatment study can be a formal clinical trial or study performed on a relatively large group of subjects wherein the study is performed according to set guidelines (*e.g.*, governmental regulations). However, the treatment study can also be a preclinical study, a field trial of a plant population or even an informal study by a scientist, veterinarian or a physician of the effects of a treatment on relatively few subjects. In a treatment study, the subjects are divided into several (though often just two) groups. These may represent different doses ranges or simply the treated and the untreated subjects. In the study, the random variable is measured after treatment. It may also be measured before treatment if it is a change in the variable over time that is being investigated (*e.g.*, bone mineral density or blood pressure). It is preferable that subjects are not undergoing any other treatments for their pathological condition. However, if such a constraint is unreasonable, the study should be designed so that subjects in both treated and untreated groups are undergoing the same alternative treatment. The subjects of the treatment study can be conducted with any type of organism, including, for example, animals (including humans), plants, bacteria and viruses.

A "biological condition" refers to the condition, susceptibility or resistance of the organism upon which the study seeks to determine whether the treatment procedure has an effect. Typically, the biological condition is a physical or physiological condition of the organism. For example, in some instances the biological condition is a pathological condition (*i.e.*, a physiological state that normally does not exist, such as a disease for example). Pathological conditions typically studied with the methods of the invention are those with a minimal environmental variance (*e.g.*, high cholesterol levels in

serum), although this is not required. Examples of pathological conditions include AIDS, arteriosclerosis, cancer, and diabetes, elevated blood pressure, elevated serum cholesterol level or psychosis. A biological condition can be the biological susceptibility or resistance of a subject. For example, the treatment study can involve an analysis of the effect of certain treatments on the susceptibility of a plant to an herbicide or susceptibility of a plant to frost damage. Alternatively, the study can be directed towards an organism defense response (*i.e.*, resistance) to some type of insult, for example.

A "random variable," either discrete or continuous, can be any biological, physiological or biochemical endpoint measured or observed, particularly in the setting of a clinical study. This includes measured and observed effects of treatments, the changes in those observations and measurements over the course of time (the so –called natural history), or any other intervention that may alter traits, signs or symptoms. Examples of random variables include pathological conditions susceptible to treatment with, *e.g.*, pharmaceutical compounds; biologics, including recombinantly produced proteins; surgical techniques; restrictive diets; and behavioral therapy. For example, serum concentrations of cholesterol, height, body mass, are all continuous random variables. This notion can extend to discrete variables such as the presence or absence of a physical trait or symptom which include, for example, nevi on the skin, cysts in the liver, particular antibodies in the serum, the degree of swollenness of the joints, the number of affected joints, or the number of hallucinations in a psychotic episode.

A "test parameter" is the characteristic that is measured or observed to determine the effect or efficacy (or lack thereof) of the treatment procedure being evaluated and is utilized to determine whether there is a statistically significant difference in the treatment and control protocols. The test parameter can be a random variable. Typically, the test parameter is expressed in quantitative terms, although in some instances the test parameter can be evaluated in qualitative terms. The nature of the test parameter varies according to the biological condition being studied. If the biological condition is a disease, the test parameter provides a measure for the status of the disease. For example, if the biological condition is AIDS, the test parameter can be the concentration of HIV in the blood of a subject. If the biological condition is

arteriosclerosis, the test parameter can be serum cholesterol concentration.

The term "variance" refers to variation, scatter, spread or dispersion about the arithmetic mean. Schematically, the variance is the mean value of the squared deviations (Armitage, P., STATISTICAL METHODS IN MEDICAL RESEARCH, Blackwell Scientific, Oxford, United Kingdom (1971)). A large variance indicates large deviations from the arithmetic mean. For example, if cholesterol level is the test parameter being measured, a mean cholesterol level is determined. The variance represents the average squared deviation of all cholesterol levels relative to the mean. Other statistical measures of spread or dispersion about a mean can also be used. Typically, the distribution of the test parameter takes the shape of a bell-shaped or a normal (Gaussian) curve. Pictorially, the invention decreases the variance and thus narrows the bell-shape of the normal curve or, described mathematically, the distribution becomes leptokurtic.

Typically, the variance is due to dissimilar effects on the subjects that influence the biological condition being analyzed by statistical methods, *e.g.*, genetic, environmental and measurement variables. For example, in most treatment studies, because the same methods are used to measure the biological condition among the entire population being studied, the variance is due to genetic differences between individual subjects and the environment in which the subjects live. Examples of environmental influences include diet, sleep patterns, geographical location and culture.

A "polymorphism" refers to the occurrence of two or more genetically determined alternative sequences or alleles in a population generally said to be occurring at a frequency of greater than 0.1%. A polymorphic marker or site is the locus at which genetic divergence occurs. Preferred markers have at least two alleles, each occurring at frequency of greater than 1% in a selected population. A polymorphic locus can be as small as one base pair. Such a locus is referred to as a single nucleotide polymorphism or simply SNP.

Polymorphic markers include restriction fragment length polymorphisms, variable number of tandem repeats (VNTR's), hypervariable regions, minisatellites, dinucleotide repeats, trinucleotide repeats, tetranucleotide repeats, simple sequence

repeats, and insertion elements such as Alu. The first identified allelic form is arbitrarily designated as the reference form and other allelic forms are designated as alternative or variant alleles. The allelic form occurring most frequently in a selected population is sometimes referred to as the wildtype form or allele and the other forms referred to as mutant forms or alleles. Diploid organisms can be homozygous or heterozygous for allelic forms. A diallelic polymorphism has two forms. A triallelic polymorphism has three forms.

A "single nucleotide polymorphism" occurs at a polymorphic site that is occupied by a single nucleotide, which is the site of variation between allelic sequences. The site is usually preceded by and followed by highly conserved sequences of the allele (*e.g.*, sequences that vary in less than 1/100 or 1/1000 members of the populations).

A single nucleotide polymorphism (SNP) usually arises due to substitution of one nucleotide for another at the polymorphic site. A transition is the replacement of one purine by another purine or one pyrimidine by another pyrimidine. A transversion is the replacement of a purine by a pyrimidine or vice versa. Single nucleotide polymorphisms can also arise from a deletion of a nucleotide or an insertion of a nucleotide relative to a reference allele.

A "polymorphic profile" refers to one or more polymorphic forms for which a subject is characterized. A polymorphic form is characterized by identifying which nucleotide(s) is (are) present at a polymorphic site in a nucleic acid sample acquired from a subject. The profile includes at least one polymorphic form and preferably includes a plurality of polymorphic forms, such as at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 polymorphic forms or more. Polymorphic profiles are similar when the polymorphic profiles being compared share at least one polymorphic form at least one polymorphic site. Typically, similar polymorphic profiles share identity of polymorphic forms in at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% in at least 10, 20, 30, 40, 50, 60, 70, 100, or 500 polymorphic sites. Polymorphic forms are identical if the nucleotide(s) at a particular polymorphic site are the same. Thus, two polymorphic profiles each including 10 polymorphic forms are 50% identical if five of the

polymorphic forms in the two profiles are identical. If the organism is diploid, then the polymorphic forms at each polymorphic site are considered to be identical in two individuals if both individuals have the same two alleles at the polymorphic site. For example, an individual having alleles a1 and a2 at polymorphic site A is considered to have the same profile as an individual having alleles a1 and a2 but not to an individual having alleles a1 and a1, or a2 and a2, or a1 and a3 and so forth.

The term "linkage" describes the tendency of genes, alleles, loci or genetic markers to be inherited together as a result of their location on the same chromosome, and can be measured by percent recombination between the two genes, alleles, loci or genetic markers.

"Linkage disequilibrium" or "allelic association" means the preferential association of a particular allele or genetic marker with a specific allele, or genetic marker at a nearby chromosomal location more frequently than expected by chance (see, for example, Weir, B., Genetic Data Analysis, Sinauer Associate Inc., 1996). For example, if locus X has alleles a and b, which occur equally frequently, and linked locus Y has alleles c and d, which occur equally frequently, one would expect the combination ac to occur with a frequency of 0.25. If ac occurs more frequently, then alleles a and c are in linkage disequilibrium. Linkage disequilibrium may result from natural selection of certain combination of alleles or because an allele has been introduced into a population too recently to have reached equilibrium with linked alleles.

A marker in linkage disequilibrium can be particularly useful in detecting susceptibility to disease (or other phenotype) notwithstanding that the marker does not cause the disease. For example, a marker (X) that is not itself a causative element of a disease, but which is in linkage disequilibrium with a gene (including regulatory sequences) (Y) that is a causative element of a phenotype, can be detected to indicate susceptibility to the disease in circumstances in which the gene Y may not have been identified or may not be readily detectable.

"Haplotype" refers to a collection of polymorphic markers either in close physical proximity on a single chromosome, or unlinked physically but associated together, which confers a biologic property or association with a phenotype.

A "nucleic acid" is a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, including known analogs of natural nucleotides unless otherwise indicated.

The term "genotype" as used herein broadly refers to the genetic composition of an organism, including, for example, whether a diploid organism is heterozygous or homozygous for one or more alleles of interest.

The term "primer" refers to a single-stranded oligonucleotide capable of acting as a point of initiation of template-directed DNA synthesis under appropriate conditions (i.e., in the presence of four different nucleoside triphosphates and an agent for polymerization, such as, DNA or RNA polymerase or reverse transcriptase) in an appropriate buffer and at a suitable temperature. The appropriate length of a primer depends on the intended use of the primer but typically ranges from 15 to 30 nucleotides, although shorter or longer primers can also be used. Short primer molecules generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. A primer need not reflect the exact sequence of the template but must be sufficiently complementary to hybridize with a template. The term "primer site" refers to the area of the target DNA to which a primer hybridizes. The term "primer pair" means a set of primers including a 5' upstream primer that hybridizes with the 5' end of the DNA sequence to be amplified and a 3', downstream primer that hybridizes with the complement of the 3' end of the sequence to be amplified.

The term "nucleic acid probe" refers to a nucleic acid molecule that binds to a specific sequence or sub-sequence of another nucleic acid molecule. A probe is preferably a nucleic acid molecule that binds through complementary base pairing to the full sequence or to a sub-sequence of a target nucleic acid. Probes can bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. The probes are typically directly labeled as

with isotopes, chromophores, lumiphores, chromogens, or indirectly labeled such as with biotin to which a streptavidin complex can later bind. By assaying for the presence or absence of the probe, the presence or absence of the select sequence or sub-sequence can be detected.

A "label" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, dioxigenin, or haptens and proteins for which antisera or monoclonal antibodies are available (*e.g.*, by incorporating a radio-label into the peptide, and used to detect antibodies specifically reactive with the peptide). A label often generates a measurable signal, such as radioactivity, fluorescent light or enzyme activity, which can be used to quantitate the amount of bound label.

A "labeled nucleic acid probe" is a nucleic acid probe that is bound, either covalently, through a linker, or through ionic, van der Waals or hydrogen bonds to a label such that the presence of the probe can be detected by detecting the presence of the label bound to the probe.

The phrase "selectively hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (*e.g.*, total cellular) DNA or RNA. The phrase "stringent hybridization conditions" refers to conditions under which a probe hybridizes to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and are different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10 °C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes

complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). Stringent conditions are those in which the salt concentration is less than about 1.0 sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30 °C for short probes (e.g., 10 to 50 nucleotides) and at least about 60 °C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions can also be achieved with the addition of destabilizing agents as formamide. If degenerate hybridization is desired, less than stringent conditions are necessary. For example, if single nucleotide mismatching is preferred, hybridization conditions will be relaxed with lower temperatures and higher salt content.

The term "statistical correlation" refers to a statistical association between two variables or parameters as measured by any statistical test including, for example, chi-squared analysis, ANOVA or multivariate analysis. The correlation between a polymorphic form of DNA and a random variable or test parameter is considered statistically significant if the probability of the result happening by chance (the P-value) is less than some predetermined level (*e.g.*, 0.05). The term "statistically significant difference" refers to a statistical confidence level, P, that is < 0.05, preferably < 0.01, and most preferably < 0.001.

A "drug" or "pharmaceutical agent" means any substance used in the prevention, diagnosis, alleviation, treatment or cure of a disease. The terms include a vaccine, for example.

The term "subject" or "individual" typically refers to humans, but also to mammals and other animals, multicellular organisms such as plants, and single-celled organisms or viruses. "Tissue" means any sample taken from any subject, preferably a human. Tissues include blood, saliva, urine, biopsy samples, skin or buccal scrapings, and hair.

The term "patient" refers to both human and veterinary subjects.

II. General

The present invention provides methods, computer programs and computerized systems useful for designing treatment studies and for evaluating the efficacy of various types of treatment procedures (*e.g.*, clinical trials) as a function of the genotype of a subject. The methods of the invention are designed to control for underlying genetic factors that may influence the response to a treatment. The present invention is based, in part, on the insight that controlling, either directly or indirectly, genetic factors that influence a patient's response to treatment can greatly increase the power of the clinical trial. Some methods are designed to reduce the genetic diversity of the patient population so as to increase the probability of individuals sharing the same alleles at genes involved in response to the treatment. In cases where polymorphisms (usually in genes) are known to be associated with or cause differences in response to the treatment, these polymorphisms can be used directly in the design of the clinical trial.

For example, the invention provides methods for reducing the variance in the biological condition or phenotype of interest by controlling for genetic factors influencing that phenotype. In the context of a clinical trial, the phenotype of interest is the response to a treatment. Genetic factors can be controlled in a number of different ways but the principle underlying the methods of the invention can be illustrated by an example. If the test parameter is measured in two groups, the first (which is of size n) is treated and the second (of size m) is untreated, the mean and variance of these samples can be calculated in the standard way (see Armitage & Berry, Statistical Methods in Medical Research, Blackwell Science, 1995.) Thus, for instance, in an example the mean and variance of the treated group are μ_1 and s_1^2 respectively, and the mean and variance of the untreated group are μ_2 and s_2^2, respectively. Then an approximate confidence interval at $\alpha\%$ (where, for example $\alpha = 0.95$) for the difference in response between the two groups is given as,

$$\mu_1 - \mu_2 \pm Z_{\alpha/2}\sqrt{\frac{s_1^2}{n} + \frac{s_2^2}{m}}$$

where $Z_{\alpha/2}$ is the value of the standard normal distribution that is exceeded by chance in $\alpha/2\%$ of cases.

Hence, any method that decreases the variance in either sample (*i.e.*, which decreases s_1^2 or s_2^2) necessarily decreases the size of the confidence interval. Alternatively, when the variance of one of both of the samples is decreased, the size of the confidence interval can be held constant with fewer patients enrolled in the trial (*i.e.*, n and/or m can be reduced). Thus, reducing the variance in response can lead either to greater certainty of a difference (here encapsulated by a smaller confidence interval) or in a reduced sample size for the same statistical power. The variance can be reduced in a number of different ways as described in the following sections.

A. Selective enrollment of patients. One approach is to control for potentially confounding factors by increasing the homogeneity of the population. In the context of genetics, a set of polymorphic markers can be examined in a large group of subjects and those with similar polymorphic profiles enrolled in the treatment study. Incorporating genetic factors (represented by the polymorphic profile) into the inclusion/exclusion criteria of a treatment study allows an experimenter to reduce the variance in response due to underlying genetic factors.

B. Division of patient population into genetically homogenous subsets. A second approach is to categorize individuals into subsets depending on how similar the polymorphic profiles are to one another. Within each subset, subjects are randomly allocated into treatment or control subpopulations, as they are in a standard clinical trial for example. This method of dividing the subjects creates subsets that are genetically more homogenous than a random sample of the same size. This design is equivalent to conducting several small, independent treatment studies each of which contains patients that have more similar polymorphic profiles than expected by chance. Many environmental variables can be manifestations of underlying genetic factors. By examining genetic polymorphisms directly, it is possible not only to reduce variance due to genetic factors that are not directly observable, but also to improve the stratification based on environmental factors that are acting as surrogates for the underlying genetics factors that control them. As

used herein, stratification refers to the division of the sample into subsets that are more similar than expected by chance for a given factor.

C. Matching of patients for their polymorphic profiles. An alternative approach is to match the subjects in the treatment and control groups. That is, pairs of individuals with similar polymorphic profiles are sought and one is allocated to the treatment group while the other is placed in the control group. In this way, the difference in response of each pair can be examined where the pairs have been matched for their underlying genetics. In the same manner as described in section B above, matching on the basis of genetic factors can control new, previously unknown causes of variance due to genetic factors and also provide greater discriminatory power when matching by environmental factors that have an underlying genetic cause.

D. Using genes known to influence response in the design of clinical trials. When one or more known polymorphisms is known to be associated with the response to treatment, these may be used directly to allocate patients into treatment and control groups. In the simplest case where a subject's polymorphic profile indicates whether or not they will respond to the treatment, this information can be used as an exclusion/inclusion criterion at the time of enrollment, thus reducing the sample size needed to observe a given level of response. Alternatively, all subjects can be enrolled in the treatment study with the treatment non-randomly assigned. For example, those known to be non-responders by their polymorphic profile can be treated according to a control procedure (*e.g.*, administered a placebo), while those who deemed responders from their polymorphic profile can be given the treatment procedure (*e.g.*, administered a drug). This maximizes the difference in response between treatment and control groups. Conversely, non-responders can be given the treatment and responders the treatment. In this scenario, the minimum difference between treated and untreated subjects can be evaluated.

E. Use of genes known to influence response to determine dosing. When one or more known polymorphisms is known to be associated with response to treatment, this information may be used to allocate the most appropriate dose to subjects enrolled in a treatment study such as a clinical trial. The polymorphic profiles of patients can determine the degree of response of individuals to the treatment. In this way, it may be possible to

allocate different doses to different patient depending on their polymorphic profiles. For example, if a treatment potentially has side effects, it will be desirable to administer the minimum efficacious dose. This can vary for subjects with different polymorphic profiles.

F. Post-trial analysis and sub-division. The present invention can also be used after the completion of a treatment study such as a clinical trial. Data obtained from such a treatment study are re-analyzed on subsets of the treated and control populations selected for similarity of a polymorphic profile to each other. The reanalysis of data is carried out on subsets of individuals sharing a similar polymorphic profile and indicates whether the treatment reaches statistical significance on individuals having that profile. If the profile contains one or more polymorphic forms associated in some way with the biological condition of interest (*e.g.*, disease), the treatment may reach statistical significance on the subpopulations when it does not on the initial treatment populations. If the profile does not contain such polymorphic DNA forms, then the re-analysis of data also shows a lack of statistical significance.

At this point, a further re-analysis is performed in which further subpopulations of individuals from treated and control populations are selected for similarity to a second polymorphic profile. Because the individuals have already been characterized for polymorphic profile, the second re-analysis can be performed without further experimental work in a highly automated and iterative fashion. Again, the second analysis indicates whether the treatment reaches statistical significance on the individuals having similarity to the polymorphic profile by which subpopulations are selected in the second analysis.

Subsequent rounds of analysis can be performed according to the same principles without further experimental work. A suitably programmed computer can perform thousand, millions or billions of cycles of analysis in which different subpopulations of individuals are selected based on similarity to different polymorphic profiles. Performing multiple tests typically requires a re-evaluation of the p-value at which a result is declared to be statistically significant to control the rate of false positive results. If after exhaustive analysis, statistical significance is not reached for any polymorphic profile, one can conclude with increased confidence that the treatment

procedure (*e.g.*, administration of a drug) being tested is unlikely to be effective in any significant portion of the population, and that further research is not justified. If, however, statistical significance is reached for a particular polymorphic DNA profile, at least two conclusions follow. First, in the case of a clinical trial on a drug that the drug is effective in at least a portion of the population, and further development of the drug may well be justified. Second, one knows the portion of the general population in which the drug is effective, this portion being defined by a polymorphic profile. This profile can be used as a diagnostic to identify patients appropriate for treatment when the decision to treat or a choice of treatments is made.

As an example of a method of the invention, a clinical trial can be carried out as follows:

1. Identification and choice of polymorphisms.

A set of polymorphisms is identified that allow the division of the patient cohort into sub-groups. These polymorphisms may be known to be involved in the test parameter (*e.g.*, the phenotype or endpoint) that is to be measured or can be chosen at random. (In the latter case, the genetic sub-groups may show identical results with respect to the phenotype of interest. This implies the method of grouping does not decrease the variance in the endpoint and the population can be re-analyzed as a whole. Thus, stratification by using genetic data does not have a deleterious effect on the experiment or trial, even in cases where it does not influence the outcome).

2. Genotyping of the cohort.

Some or all of the markers are genotyped in the entire cohort of patients enrolled in the clinical trial. These data are then used either as inclusion/exclusion criteria (see 3a below) or to divide the cohort into subgroups (see 3b below).

3a. Inclusion/exclusion of patients using genetic information.

If some or all of the polymorphisms are known to influence the test

parameter that is to be measured, it may be appropriate to exclude individuals when it is known, *a priori*, they will present a particular phenotype or endpoint. In the context of a clinical trial, this can represent excluding those individuals who, by information gained from the set of polymorphisms examined, will not respond to the therapy.

3b. Division of the clinical trial into subgroups.

A metric is used to determine the genetic similarity of patients in the cohort. This information is used to divide the population into subgroups that have greater genetic similarity than might be expected by chance. That is, the subgroups are genetically more homogenous than a random subset of the same size.

The precise method of measuring similarity will depend on the number and type of markers used. In the simplest case, the number of markers at which two individuals have the same alleles can be used to determine similarity. Many other more complex metrics can be employed that, for example, giving extra weight to markers known to be particularly informative or that influence the test parameter of interest.

By altering the method of determining genetic similarity, an experimenter can control the number of subgroups that need to be formed. For N individuals, this can range from 1 (the entire population) to N (each individual is in a separate subgroup). Practical as well as scientific reasons are considered in determining how many subgroups are optimal for a given experiment or trial. With the methods of the invention, groups can be merged at a later time.

4. Allocation of treatment within the genetic subgroups.

When the patients have been grouped into genetic subgroups based on information from the set of polymorphism described in 1, several strategies are available for conducting a treatment study such as a clinical trial.

One method is to randomize the treatment and placebo within each subgroup. This is similar to treating each subgroup as a separate experiment or clinical trial. Results of each subgroup may be analyzed separately or may be pooled and then analyzed.

Alternatively, treatment can be non-randomly allocated within the subgroups. This may be appropriate, for example, when the polymorphisms are known to be associated with the outcome or endpoint of interest. For example, in the context of a clinical trial, if there are only two subgroups and one of the subgroups is known to contain high responders and the other low responders to a treatment, allocating the treatment to the first group and the placebo to the second group maximizes the difference between response for treated and untreated individuals. Conversely, allocating the placebo to the first group and the treatment to the second group shows the minimum difference between treated and untreated individuals. Which of these approaches is most appropriate depends on the exact objective of the experiment or clinical trial.

5. Use of information from one experiment in the design of subsequent studies.

The utility of stratifying by using a set of genetic polymorphisms can be re-assessed through successive experiments of clinical trials. Uninformative polymorphisms can be dropped and new polymorphisms added to increase the usefulness of the set as a whole. Use of these polymorphisms in subsequent treatment studies or clinical trials leads to greater reproducibility of results and the need for enrolling fewer subjects in replication studies.

By identifying and correlating polymorphisms to a particular effect of a drug, and thus reducing the variance due to genetic factors, a clinician can devise clinical trials that involve fewer subjects, decrease the confidence intervals, or increase the precision or discriminatory power of a given trial. The clinician can decide which of these three aspects of trial design or analysis to change while keeping the other two constant.

In addition to altering the statistic of variance which in turn can affect subject number, precision or power of a study, using analysis of polymorphic markers in a clinical trial population in a manner as disclosed herein permits, upon analysis, the identification of subsets of polymorphic markers that may correlate with either a salubrious response, unresponsiveness or excessive response to a treatment, an unwanted

or toxic response to a treatment, and may identify by virtue of unresponsiveness, a clinical subset of patients that define a "different" disease. In short, a *post facto* genetic analysis correlated with a specific clinical phenotype such as drug responsiveness or unresponsiveness can reveal different etiologic mechanisms for the disease being treated. This is especially likely in the case of ethnic differences among patients where each ethnic group has a distinctive response to a treatment. Finally, analysis of phenotypic markers can provide insight into genetic diversity of the subjects being treated allowing the clinician to alter enrollment in a drug trial to accommodate more or less genetic diversity as is scientifically prudent.

III. Methods

A. General

In the methods of the invention, members of a treated and control (untreated) population having a biological condition of interest (*e.g.*, a disease) are characterized for polymorphic profile and a test parameter that is a measure of the biological condition, assuming the members have not already been so characterized. The members in the treated population have been (or are) treated according to a treatment procedure, whereas the members of the control population have been (or are) treated according to a control procedure.

To reduce total variance in the treatment assessment or study, subpopulations from the treated and control populations are selected for similar genetic composition such that the members in the two populations have similar or identical polymorphic profiles. The polymorphic profile of the subpopulations includes one or more polymorphic forms. Typically, the polymorphic profile includes a plurality of polymorphic forms, generally at least 5, in other instances at least 10, and in still other instances at least 100, or any number there between.

To minimize genetic variance between the treated and control subpopulations, the polymorphic profiles for the two groups are selected to be similar.

This means that there is at least one common polymorphic form between the two subpopulations, although typically there are more. The polymorphic profiles for the two subpopulations are typically at least 10% identical, in some instances at least 50% identical, in still other instances greater than 75% identical, in yet other instances 90% identical or more, and in still other instances 100% identical. The analysis of phenotypic markers can provide additional insight into the genetic diversity of the subjects being treated and allows the researcher to alter the members in a study to accommodate more or less genetic diversity.

Polymorphisms can be selected in three distinct ways. First, they can be chosen at random. Second, only those polymorphisms known or suspected to be involved in the phenotype of interest (response to treatment in the case of a clinical trial) can be selected. Third, DNA polymorphism selection can be driven by identifying polymorphisms that reside in regions of the genome that have previously been shown to harbor a genetic linkage with the observable trait(s) under study. In the first case, random polymorphisms are unlikely to be directly involved in response. However, they can be used to determine the genetic similarity of patients and hence can be used to form subgroups that are more genetically homogenous than expected by chance. This strategy is particularly effective when a large number of (usually unknown) genes are involved in determining an individual's response to treatment. If there are polymorphisms known to be involved in response or to be associated with (possibly unknown) genes involved in response, then these can preferentially be used to determine subgroups of patients.

Not all polymorphisms will be equally informative or useful in determining subgroups. Factors such as the allele frequencies, whether the polymorphism is protein coding or non-coding, whether the polymorphism is in linkage disequilibrium with another polymorphism already in the polymorphic profile being used, whether the polymorphism is in linkage disequilibrium with a gene or genes known to be involved in response to treatment can all influence the utility of a given polymorphism. Note that in some cases, it may be desirable to give more weight to some polymorphisms than others in the formation of subgroups. That is, polymorphisms known to be associated with

responsiveness may be more important (and hence given more weight) than random polymorphisms.

The polymorphisms can be in genomic DNA, RNA or cDNA. While any polymorphisms can be used, those of particular import are polymorphisms in genes that encode proteins that directly or indirectly influence a biochemical pathway that is correlated with the biological condition being measured or observed. Thus, for example, if a study involves assessing the efficacy of methods for treating patients having elevated blood cholesterol levels, the polymorphic profile can be tailored to include polymorphisms located in genes known to be involved in cholesterol synthesis and metabolism.

Once appropriate subpopulations have been selected such that the subpopulations have the desired level of similarity in polymorphic profile, a determination is made whether there is a correlation between the polymorphic profile and the efficacy (or lack thereof) of the treatment method by ascertaining whether there is a statistically significant difference in a test parameter between the treated and control subpopulations, where the test parameter is a measure or is representative of the efficacy of the treatment for the biological condition shared by members of the subpopulation. A finding of a statistically significant difference, indicates that the polymorphic forms in the polymorphic profile of the treated subpopulation correlate with the biological condition (*e.g.*, the polymorphic profile is correlated with a particular disease) and that the treatment method under study is useful (or not beneficial) for treating subjects with the biological condition.

As noted above, such correlations are particularly important, for example, in clinical trials on a drug. In some instances, the correlation identifies a set of genetic markers associated with the disease and thus has diagnostic value. In other instances, the correlation identifies markers that are associated with a positive treatment result and thus are important from a therapeutic standpoint.

A statistically significant difference in a test parameter between the treatment and control subpopulations can be determined using standard methods of

statistical analysis. Methods include, for example, the analysis of variance, logistic regression, cluster analysis, non-parametric statistics, contingency table test and other standard statistical tests.

B. Repetition of Method

The polymorphic profile of the subpopulation initially selected, often do not correlate with a statistically significant difference in the test parameter that is used to measure the efficacy of treatment. In such instances, the method can be repeated with different subpopulations created by using an alternative definition or measure of genetic similarity, or by dividing the population into greater or fewer sub-populations. This reflects the fact that there will rarely be a single unique way to group patients. Indeed, for a study with N individuals, it will often be possible to form any number of sub-populations from 1 (the entire population) to N (each individual in its own sub-population). Repeating the process is often an effective way of detecting which polymorphisms within the polymorphic profile are particularly informative with respect to the test parameter of interest. Once a correlation is identified, additional cycles can be repeated using, for example, a subset of the polymorphic forms utilized in an earlier cycle to determine whether the subset might show an even greater correspondence with the test parameter and thus treatment efficacy.

Typically the polymorphic forms within a polymorphic profile evolve over time to account for a greater proportion of the genetic component of the variance. However, these polymorphic forms generally do not contribute equally. Some account for more variance than others; markers that do not correlate with differences in the treatment and control procedures are discarded from the analysis. The set of markers as a collection have value distinct from the individual markers. This collection has enduring value for understanding the genetic contribution to a distinct biological condition of interest. Individual markers can have diagnostic utility, as can the collection.

The analysis of treatment or trial data involving groups and subgroups is amenable to both parametric and non-parametric (distribution-free methods) statistical methods.

C. Treatment and Control Groups

The members of the treatment and control groups all share some biological condition upon which the study is designed to determine whether the treatment procedure has a statistically significant different effect relative to the control procedure. The members of the treatment and control groups can be essentially any type of organism including, for example, humans, non-human animals, plants, bacteria and viruses. In some instances, the members are mammals (*e.g.*, humans, primates) that are part of a clinical trial involving the testing of a pharmaceutical agent or behavioral therapy for example. The number of members in the subpopulations selected from the treatment and control group is at least one but generally is more than one, typically at least 5, in other instances at least 10, and in still other instances at least 100 or more, although any number of members between these numbers can also be selected.

In some instances, the members of the subpopulation are selected not only to have similar polymorphic profiles, but also to have other common features. Selecting on the basis of other commonalties can further reduce total variance beyond that achieved by reducing the variance attributable to genetic factors. Hence, for example, members of the treatment and control subpopulations can also be selected to have been similarly exposed to an environmental factor. Examples of such environmental factors include, but are not limited to, exposure to various agents such as radiation, chemicals, and second hand smoke; geographical location; and life style characteristics such as sleep patterns, diet, and amount of exercise. In some instances, it is useful to conduct studies using subpopulations that have not been similarly exposed to an environmental factors; such studies can be serve as a counterpoint to studies wherein the subpopulations have been selected for similar exposure to certain environmental factors. Besides environmental factors, members can also be selected to be from the same ethnic group or to share a common phenotypic trait (*e.g.*, visual acuity, height, weight, physical abnormality).

When the methods of the invention are utilized in clinical trials, typically subjects in the two groups are not undergoing any other treatments for their pathological condition. In other instances, the study is designed so that subjects in both treated and untreated groups are undergoing the same alternative treatment.

D. Treatment and Control Procedures

The types of treatment and control procedures vary according to the biological condition to which the treatment is directed. As noted above, the biological conditions can be any of a number of conditions, such as a pathological condition or simply a biological susceptibility, for example. A variety of different procedures can be performed when the biological condition is a pathological condition. In many instances, the procedures involve administering a pharmaceutical agent, including, for example: 1) administering a pharmaceutical agent to members of the treated population and giving members in the control population a placebo or nothing at all, 2) giving members of the treated population one pharmaceutical agent (or combination of pharmaceutical agents) and a different pharmaceutical agent (or combination of pharmaceutical agents) to the control members; 3) providing one quantity of a pharmaceutical agent to the treated population and a different amount to the control population, or 4) administering a pharmaceutical agent to the treatment and control populations according to different schedules.

Instead of administering a pharmaceutical agent, the treatment procedure can include some type of behavioral therapy. Examples of such therapy include, but are not limited to, a particular diet regime (*e.g.*, low fat, low sodium, high protein, or a restricted calorie diet), a prescribed exercise regime (*e.g.*, exercising for a certain time period a certain number of times a week, performing low-impact exercises, exercising to reach a target heart rate, therapies that work certain muscle groups), meditation, yoga, and stress reduction techniques. Of course, the treatment procedure can include combinations of the foregoing procedures as well. Members in control groups may not undergo therapy at all or may be treated in opposing fashion (or may already be engaged in contrary

behaviors). For example, if the treatment group is placed on a low caloric diet, members in the control group can be placed on a high caloric diet or can simply be selected for those whose normal diet already is a high caloric diet and thus is not altered.

The treatment procedure can also be directed towards a biological susceptibility or resistance rather than a pathological condition. Thus, for example, in the case of plants, plants can be treated with various agricultural agents used to affect plant growth or health (*e.g.*, fertilizer or other growth stimulants, herbicides, insecticides, and pH altering agents) to assess the effect of such agents on various susceptibilities or resistances of plants (*e.g.*, susceptibility to frost or freeze damage and resistance to herbicides). In like manner, humans or other organisms can also be treated with various agents, for example vaccines, to determine the effect of the agents on various susceptibilities or resistances.

E. Utility

The reduction in variance achieved by the methods of the invention enables researchers to selectively optimize treatment studies. For example, as the genetic variance decreases, the confidence level of the statistical analysis increases. Thus, with the methods of the invention, researchers can more confidently attribute differences in effects as seen between the treated subjects and the control subjects to the treatment administered, rather than being consequences of genetic differences between patients. Furthermore, differences between control and test groups can be appreciated sooner. This allows smaller, less costly studies to be performed that have the same statistical power as much larger studies that do not match for the underlying genetics. Alternatively, a study in which patients are matched for genetic factors will be able to detect much smaller difference in response between treated and untreated individuals than a study of the same size that ignores genetics factors. This allows for less costly studies, more rapid assessment regarding the feasibility and desirability of additional treatment studies, and ultimately, in the case of clinical trials on pharmaceutical compounds for example, allows for more rapid marketing of the pharmaceuticals.

The methods also enable more efficient treatment studies to be designed. For instance, once polymorphisms that correlate with pathological conditions have been identified, subjects that have the polymorphisms as well as the biological condition can be identified and enrolled in additional studies to analyze the effect that other treatments have on the biological condition of interest. Because subjects that will not respond to the treatment are not enrolled, fewer subjects need to be enrolled. Alternatively, if a set of polymorphisms emerges that, when matched between patients in a control and test arms of a trial, is highly correlative with the biological condition being studied, subsequent trials of the efficacy of a treatment can be tested with fewer patients regardless of response rate if the biological condition being measured has a genetic component. In addition, when polymorphisms associated with differential response are identified, it may be possible to tailor the dose a specific patient receives to be optimal given their polymorphic profile. This will be particularly important when there are unwanted side effects of the treatment and it is desirable to give the minimum efficacious dose.

Furthermore, as noted above, the treatment methods described herein permit the identification of subsets of polymorphic forms that correlate with either a favorable response or unresponsiveness to treatment, or an unwanted or toxic response to a treatment. Clinical trials on the efficacy of certain pharmaceutical treatments can identify individuals that are unresponsive to treatment and, in so doing, can in some instances result in the identification of a clinical subset of patients that define a "different" disease. Such correlations can also be used as a prognostic and/or diagnostic tool to identify subjects having or likely to acquire a disease or to select appropriate treatment procedures for a subject based upon the particular genetic composition of the subject.

Information gained from clinical trials in which patients are genotyped for a set of polymorphic genetics markers can also be used in other stages of drug discovery and development. For example, genes shown to be associated with response via the polymorphic profile of the patients may be amenable to intervention and hence represent potential drug targets. Furthermore, identification of treatments that show low efficiency (*i.e.*, many non-responders) or that have high rates of adverse events can be identified by examining the polymorphism profile of patients in early phase trials. This information

can then be used in the decision whether to take a treatment forward into large and more costly trials. For example, if non-response is associated with a polymorphism profile that is common in the general population, it may be inappropriate to use the treatment in a larger trial.

IV. Computer Systems and Programs

FIG. 1 depicts a representative computer system 10 suitable for implementing certain methods of the present invention. As shown in FIG. 1, computer system 10 typically includes a bus 12 that interconnects major subsystems such as a central processor 14, a system memory 16, an input/output controller 18, an external device such as a printer 23 via a parallel port 22, a display screen 24 via a display adapter 26, a serial port 28, a keyboard 30, a fixed disk drive 32 via storage interface 34, and a floppy disk drive 33 operative to receive a floppy disk 33A. Many other devices can be connected such as a scanner 60 via I/O controller 18, a mouse 36 connected to serial port 28, a CD ROM player 40 operative to receive a CD ROM 42, or a network interface 44. Source code to implement the present invention can be operably disposed in system memory 16 or stored on storage media such as a fixed disk 32 or a floppy disk 33A. Other devices or subsystems can be connected in a similar manner. All of the devices shown in FIG. 1 are not required to practice the present invention. The devices and subsystems can also be interconnected in different ways from that shown in FIG. 1. The operation of a computer system 10 such as that shown in FIG. 1 is readily known in the art; hence, operations of the system are not described in detail herein.

FIG. 2 is an illustration of a representative computer system 10 of FIG. 1 suitable for performing the methods of the present invention; however, FIG. 2 depicts but one example of many possible computer types or configurations capable of being used with the present invention. As depicted in FIG. 2, computer system 10 can include display screen 24, cabinet 20, keyboard 30, a scanner 60, and mouse 36. Mouse 36 and keyboard 30 are examples of "user input devices." Other examples of user input devices include, but are not limited to, a touch screen, light pen, track ball and data glove.

Mouse 36 can have one or more buttons such as buttons 37. Cabinet 20 houses familiar computer components such as floppy disk drive 33, a processor 14 and a storage means (see FIG. 1). As used in this specification "storage means" includes any storage device capable of storing data that can be used in connection with a computer system. Examples of such devices include, but are not limited to, disk drives, magnetic tape, solid-state memory and bubble memory. Cabinet 20 can include additional hardware such as input/output (I/O) interface for connecting computer system 10 to external devices such as a scanner 60, external storage, other computers or additional peripheral devices.

In some instances, system 10 includes a computer having a Pentium® microprocessor 14 that runs WINDOWS® Version 3.1, WINDOWS95® or WINDOWS98® operating system by Microsoft Corporation. However, the methods of the invention can easily be adapted to other operating systems (*e.g.*, UNIX) without departing from the scope of the present invention.

FIG. 3 is a flowchart of simplified steps in one computerized method of the invention for assessing a treatment procedure. In step 100 a database is provided that contains a plurality of designations for each member of a population that has either been treated according to a treatment procedure or a control procedure and that shares a common biological condition (the database is described further below). Hence, the population includes both treatment and control populations. One group of designations is to identify each member of the two populations. There are also typically separate designations for a polymorphic profile and a test parameter for each member of the population. Subpopulations from the treated and control populations are selected in a selection step 102 for similarity in polymorphic profile. In determining step 104, a determination is made whether there is a statistically significant difference in the test parameter between the subpopulations. A statistically significant difference indicates that the polymorphic profile of the subpopulations correlates with the biological condition and effect of treatment. Finally, in a displaying step 106, an output of the result of the determining step is displayed on an output device to facilitate the analysis. In an optional decisional step 108, if there is not a statistically significant difference in the test parameter

for the two subpopulations, the selecting step 102, the determining step 104, and the displaying step 106 are repeated using subpopulations that have a polymorphic profile that is different from that in earlier cycles.

Hence, the microprocessor in the computer system of the present invention is operatively disposed relative to the system memory, the system bus and the input/output so as to perform the foregoing functions. For example, the processor provides or receives data that comprises designations for each member of the treated and control populations, as well as designations for a polymorphic profile and a test parameter for each member of the two populations. The microprocessor is also operatively disposed to select a subpopulation from each of the treatment and control populations for similarity in polymorphic profile, determine whether there is a statistically significant difference in the test parameter between the subpopulations and display an output of the result obtained.

The computer program of the invention includes code for providing or receiving data comprising the various designations for the identity of the members of the test and control populations, their polymorphic profiles and test parameter results. The program also includes code necessary to perform the selecting, determining and displaying steps set forth above.

V. Methods for Determining Polymorphic Profiles

A. Preparation of Samples

Polymorphisms are detected in a target nucleic acid from an individual being analyzed. For assay of genomic DNA, virtually any biological sample (other than pure red blood cells) is suitable. For example, convenient tissue samples include whole blood, semen, saliva, tears, urine, fecal material, sweat, buccal, skin and hair. For assay of cDNA or mRNA, the tissue sample must be obtained from an organ in which the target nucleic acid is expressed. For example, if the target nucleic acid is a cDNA encoding cytochrome P450, the liver is a suitable source.

Many of the methods described below require amplification of DNA from target samples. This can be accomplished by *e.g.*, PCR. *See* generally, PCR TECHNOLOGY: PRINCIPLES AND APPLICATIONS FOR DNA AMPLIFICATION (ed. H.A. Erlich, Freeman Press, NY, NY, 1992); PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS (eds. Innis, *et al.*, Academic Press, San Diego, CA, 1990); Mattila, *et al.*, *Nucleic Acids Res.* **19**:4967 (1991); Eckert, *et al.*, *PCR Methods and Applications* **1**:17 (1991); PCR (eds. McPherson, *et al.*, IRL Press, Oxford); and U.S. Patent 4,683,202 (each of which is incorporated by reference for all purposes).

Other suitable amplification methods include the ligase chain reaction (LCR) (see Wu & Wallace, *Genomics* 4:560 (1989), Landegren, *et al.*, *Science* **241**:1077 (1988), transcription amplification (Kwoh, *et al.*, *Proc. Nat'l Acad. Sci. USA* **86**:1173 (1989)), and self-sustained sequence replication (Guatelli, *et al.*, *Proc. Nat'l Acad. Sci. USA* **87**:1874 (1990)) and nucleic acid based sequence amplification (NASBA). The latter two amplification methods involve isothermal reactions based on isothermal transcription, which produce both single stranded RNA (ssRNA) and double stranded DNA (dsDNA) as the amplification products in a ratio of about 30 or 100 to 1, respectively.

B. Detection of Polymorphisms in Target DNA

There are two distinct types of analysis depending whether a polymorphism in question has already been characterized. The first type of analysis is sometimes referred to as *de novo* characterization. This analysis compares target sequences in different individuals to identify points of variation, *i.e.*, polymorphic sites. The second type of analysis involves determining which form(s) of a characterized polymorphism are present in individuals under test. There are a variety of suitable procedures for determining polymorphic forms and thus polymorphic profiles, including, for example, the methods that follow.

1. Allele-Specific Hybridization (ASH)

ASH technology is based on the stable annealing of a short, single-stranded, oligonucleotide probe to a completely complementary single-strand target nucleic acid. Hybridization is detected from a radioactive or non-radioactive label on the probe. For each polymorphism, two or more different probes are designed to have identical DNA sequences, except at the polymorphic nucleotides. Each probe has exact homology with one allele sequence so that the complement of probes can distinguish all the alternative allele sequences. With appropriate probe design and stringency conditions, a single-base mismatch between the probe and target DNA prevents hybridization. In this manner, only one of the alternative probes hybridizes to a target sample that is homozygous for an allele (an allele is defined by the DNA homology between the probe and target). Samples containing DNA that is heterozygous for two alleles hybridize to both of two alternative probes. Details regarding ASH are described, for example, by Saiki, *et al., Nature* **324**:163-166 (1986); Dattagupta, EP 235,726; Saiki, WO 89/11548; and in U.S. Patent 5,468,613.

2. Restriction Fragment Length Polymorphisms (RFLP)

The phrase "restriction fragment length polymorphism" or "RFLP" refers to inherited differences in restriction enzyme sites (for example, caused by base changes in the target site), or additions or deletions in the region flanked by the restriction enzyme site that result in differences in the lengths of the fragments produced by cleavage with a relevant restriction enzyme. A point mutation leads to either longer fragments if the mutation is within the restriction site or shorter fragments if the mutation creates a restriction site. Additions and transposable elements lead to longer fragments and deletions lead to shorter fragments.

An RFLP can be used as a genetic marker in the determination of segregation of alleles with quantitative phenotypes. In one embodiment of the invention, the restriction fragments are linked to specific phenotypic traits. More specifically, the presence of a particular restriction fragment can be used to predict the prevalence of a specific phenotypic trait.

3. Direct-Sequencing

Polymorphisms can be analyzed directly using the traditional dideoxy-chain termination method or the Maxam -Gilbert method (*see* Sambrook, *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL (2nd Ed., CSHP, New York 1989); and Zyskind *et al.*, RECOMBINANT DNA LABORATORY MANUAL, (Acad. Press, 1988)). Other nucleic acid sequencing methods, including, but not limited to, fluorescence-based techniques (U.S. Patent No. 5,171,534), mass spectroscopy (U. S. Patent No. 5,174,962) and capillary electrophoresis (U.S. Patent No. 5,728,282) can also be used.

4. Drag-Tagging Oligonucleotides for Electrophoresis

Oligonucleotides having additional chemical moieties that cause differential mobilities in an electrophoretic separation system can be used in analyzing polymorphisms. The addition of a molecular species increases the apparent molecular weight of a piece of amplified DNA, even DNA having only a single nucleotide polymorphism present. The added species, or drag tags, can be attached covalently or non-covalently to the nucleic acid, either before or after labeling (for visualization of the electrophoretic band). Any charge associated with the added species is blocked or neutralized so that nucleic acid mobility remains dependent on size and not charge.

Any of a number of different moieties can be attached to a nucleic acid to form a plurality of different sized amplification products. Examples of such moieties include, but are not limited to, phosphate monomers, acrylamide and polypeptides. Thus, for instance, prior to amplification of a polymorphic stretch of DNA, phosphate monomer units can be attached to each nucleotide monomer that is to be used in the PCR reaction. For example, assume one phosphate monomer is added to dATP; two phosphate monomers are added to dCTP; three phosphate monomers are added to dGTP; and four phosphate monomers are added to dTTP. The resulting amplified polymorphic nucleic acids contain different amounts of phosphate monomers depending on the nucleotide content. Hence, while the amplified products have the same numbers of base pairs, the different polymorphic forms nonetheless may be size separated on an electrophoretic gel due to differences in phosphate monomer content. In a variation of this general approach, the

monomer units are added after amplification to specific nucleotides or to non-amplified nucleic acids prior to separation on the basis of size (*e.g.*, by capillary electrophoresis).

5. Isozyme Markers

Other embodiments include identification of isozyme markers and allele-specific hybridization. Isozymes are a group of enzymes that catalyze the same reaction but vary in physical properties resulting from differences in amino acid sequence (and hence nucleic acid sequence). Some isozymes are multimeric enzymes containing slightly different subunits. Other isozymes are either multimeric or monomeric but have been cleaved from the proenzyme at different sites in the amino acid sequence. Nucleic acid variation of isozymes can be determined by hybridizing primers that flank a variable portion of an isozyme nucleic acid sequence to target nucleic acids contained in a sample obtained from an organism. The variable region is amplified and sequenced. From the sequence, the different isozymes are determined and linked to phenotypic characteristics.

6. Amplified Variable Sequences

Amplified variable sequences of the genome and complementary nucleic acid probes also can be used as polymorphic markers. The phrase "amplified variable sequences" refers to amplified sequences of the genome that exhibit high nucleic acid residue variability between members of the same species. All organisms have variable genomic sequences and each organism (with the exception of a clone) has a different set of variable sequences. The presence of a specific variable sequence can be used to predict phenotypic traits. A variable sequence of DNA can be amplified (*e.g.*, utilizing the amplification techniques listed above) by template-dependent extension of primers that hybridize to flanking regions of the DNA obtained from a subject. The amplified products can then be sequenced.

7. Allele-Specific Primers and Hybridization

An allele-specific primer hybridizes to a site on target DNA overlapping a polymorphism and only primes amplification of an allelic form to which the primer exhibits perfect complementarity. This primer is used in conjunction with a second

primer that hybridizes at a distal site. Amplification proceeds from the two primers and produces a detectable amplified product that can be characterized for the particular allelic form present in a nucleic acid sample. *See, e.g.,* Gibbs, Nucleic Acid Res. 17:2427-2448 (1989) and WO 93/22456.

8. Single-Strand Conformation Polymorphism Analysis

Alleles of target sequences can be differentiated using single-strand conformation polymorphism analysis, which identifies base differences by alteration in electrophoretic migration of single-stranded PCR products, (*see, e.g.,* Orita, *et al., Proc. Nat'l Acad. Sci. USA* 86:2766-2770 (1989). Typically, amplified PCR products are denatured (*e.g.*, according to known chemical or thermal methods) to form single-stranded amplification products that can refold or form secondary structures, depending in part upon the base sequence of the product. The different electrophoretic mobilities of single-stranded amplification products can be related to base-sequence difference between alleles of target sequences.

9. Self-sustained Sequence Replication

Polymorphisms can also be identified by self-sustained sequence replication. In this approach, target nucleic acid sequences are amplified (replicated) exponentially *in vitro* under isothermal conditions using three enzymatic activities involved in retroviral replication: (1) reverse transcriptase, (2) RNase H, and (3) a DNA-dependent RNA polymerase (Guatelli, *et al., Proc. Natl. Acad. Sci. USA* 87:1874 (1990)). By mimicking the retroviral strategy of RNA replication by means of cDNA intermediates, cDNA and RNA copies of the original target are accumulated.

10. Arbitrary Fragment Length Polymorphisms (AFLP)

Arbitrary fragment length polymorphisms (AFLP) can also be used as polymorphisms (Vos, *et al., Nucl. Acids Res.* 23:4407 (1995)). The phrase "arbitrary fragment length polymorphism" refers to selected restriction fragments that are amplified before or after cleavage by a restriction endonuclease. The amplification step permits

easier detection of specific restriction fragments as compared to determining the size of all restriction fragments and comparing the sizes to a known control.

AFLP allows the detection of a large number of polymorphic markers (*see, supra*) and has been used for genetic mapping of plants (Becker, *et al., Mol. Gen. Genet.* 249:65 (1995); and Meksem, *et al., Mol. Gen. Genet.* 249:74 (1995)) and to distinguish among closely related bacterial species (Huys, *et al., Int'l J. Systematic Bacteriol.* 46:572 (1996)).

11. Simple Sequence Repeats (SSR)

SSR methods are based upon high levels of di-, tri- or tetra-nucleotide tandem repeats within a genome. Dinucleotide repeats have been reported to occur in the human genome as many as 50,000 times with n varying from 10 to 60 (Jacob, *et al., Cell* 67:213 (1991)). The dinucleotide repeats have also been found in higher plants (Condit & Hubbell, *Genome* 34:66 (1991)).

SSR data is generated by hybridizing primers to conserved regions of the genome that flank the SSR region. The dinucleotide repeats between the primers are amplified by PCR. The resulting amplified sequences are then electrophoresed to determine the size, and therefore the number, of di-, tri- and tetra-nucleotide repeats.

12. Denaturing Gradient Gel Electrophoresis

Amplification products generated using the polymerase chain reaction can be analyzed through the use of denaturing gradient gel electrophoresis. Different alleles are identified based on the different sequence-dependent melting properties and electrophoretic migration of DNA in solution. Erlich, ed., PCR TECHNOLOGY, PRINCIPLES AND APPLICATIONS FOR DNA AMPLIFICATION, (W.H. Freeman and Co, New York, 1992), Chapter 7.

13. Single base extension methods

Polymorphisms can also be detected by single base extension. A primer is designed to hybridize to a target sequence so that the 3' end of the primer immediately abuts but does not overlap a polymorphic site. The target sequence is then contacted with

primer and at least one nucleotide (typically labelled), that is complementary to the base occupying the polymorphic site in one allelic form. If that allelic form is present, then the primer is extended and becomes labelled. In some methods, biallelic polymorphic sites are analyzed by including two differentially labelled dideoxynucleotides respectively complementary to bases occupying the polymorphic site in first and second allelic forms of the target. Analysis of label present in the extended primer indicates whether one or both of the allelic forms are present in a target sample.

C. High Throughput Screening

In some instances, identification of polymorphisms is done by high throughput screening. In one embodiment, high throughput screening involves providing a library of polymorphic forms of DNA including RFLPs, AFLPs, isozymes, specific alleles and variable sequences, including SSR. Such "libraries" are then screened against genomic DNA from the subjects in the treatment study. Once the polymorphic alleles of a subject have been identified, a link between the polymorphic DNA and the treatment effect can be determined through statistical associations.

Such high throughput screening can be performed in many different formats. For example, for those methods involving hybridization reactions, hybridization can be performed in a 96-, 324-, or a 1024-well format or in a matrix on a silicon chip. In a well-based format, a dot blot apparatus is used to deposit samples of fragmented and denatured genomic DNA on a nylon or nitrocellulose membrane. After cross-linking the nucleic acid to the membrane, either through exposure to ultra-violet light if nylon membranes are used or by heat if nitrocellulose is used, the membrane is incubated with a labeled hybridization probe. The membranes are washed extensively to remove non-hybridized probes and the presence of the label on the probe is determined.

The labels are incorporated into the nucleic acid probes by any of a number of methods well known to those of skill in the art. In some instances, a label is simultaneously incorporated during the amplification procedure in the preparation of the nucleic acid probes. Thus, for example, polymerase chain reaction (PCR) with labeled

primers or labeled nucleotides provide labeled amplification product. In another embodiment, transcription amplification using a labeled nucleotide (*e.g.*, fluorescein-labeled UTP and/or CTP) incorporates a label into the transcribed nucleic acid probes.

Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, radioisotopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include biotin for staining with labeled streptavidin conjugate, magnetic beads, fluorescent dyes (*e.g.*, fluorescein, Texas red, rhodamine, green fluorescent protein, and the like), radiolabels (*e.g.*, ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (*e.g.*, polystyrene, polypropylene, latex, *etc.*) beads. Patents teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

Methods for detecting such labels are also well known to those of skill in the art. Thus, for example, radiolabels are detected using photographic film or scintillation counters and fluorescent markers are detected using a photodetector to detect emitted light. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

A number of well-known robotic systems have been developed for high throughput screening, particularly in a 96 well format. These systems include automated workstations like the automated synthesis apparatus developed by Takeda Chemical Industries, LTD. (Osaka, Japan) and many robotic systems utilizing robotic arms (Zymate II, Zymark Corporation, Hopkinton, Mass.; Orca, Hewlett-Packard, Palo Alto, Calif.) that mimic the manual synthetic operations performed by a chemist. Any of the above devices are suitable for use with the present invention. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art.

In addition, high throughput screening systems themselves are commercially available (*see, e.g.*, Zymark Corp., Hopkinton, MA; Air Technical Industries, Mentor, OH; Beckman Instruments, Inc. Fullerton, CA; Precision Systems, Inc., Natick, MA, etc.). These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate or membrane in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. The manufacturers of such systems provide detailed protocols the various high throughput.

D. Solid-Phase Arrays

Polymorphic forms of DNA can also be identified by hybridization to nucleic acid arrays, some examples of which are described by WO 95/11995 (incorporated by reference in its entirety for all purposes). In one variation of the invention, solid phase arrays are adapted for the rapid and specific detection of multiple polymorphic nucleic acids. Typically, a nucleic acid probe is linked to a solid support and a target nucleic acid is hybridized to the probe. Either the probe, or the target, or both, can be labeled, typically with a fluorophore. If the target is labeled, hybridization is detected by detecting bound fluorescence. If the probe is labeled, hybridization is typically detected by quenching of the label by the bound nucleic acid. If both the probe and the target are labeled, detection of hybridization is typically performed by monitoring a color shift resulting from proximity of the two bound labels.

The construction and use of solid phase nucleic acid arrays to detect target nucleic acids has been described extensively in the literature. See, Fodor, *et al., Science* **251**:767 (1991); Sheldon, *et al., Clin. Chem.* 39(4):718 (1993); Kozal, *et al., Nature Medicine* 2(7):753 (1996) and Hubbell, U.S. Pat. No. 5,571,639. See also, Pinkel, *et al.*, PCT/US95/16155 (WO 96/17958). In addition to being able to design, build and use probe arrays using available techniques, one of skill is also able to order custom-made

arrays and array-reading devices from manufacturers specializing in array manufacture. For example, Affymetrix in Santa Clara CA manufactures DNA VLSIP™ arrays.

It will be appreciated that probe design is influenced by the intended application. For example, where several probe-target interactions are to be detected in a single assay, *e.g.*, on a single DNA chip, it is desirable to have similar melting temperatures for all of the probes. Accordingly, the lengths of the probes are adjusted so that the melting temperatures for all of the probes on the array are closely similar (different lengths for different probes may be needed to achieve a particular Tm where different probes have different GC contents). Although melting temperature is a primary consideration in probe design, other factors are optionally used to further adjust probe construction.

E. Capillary and Microchannel Plate Electrophoresis

As described above, certain methods for identifying polymorphisms involve size based separations (*e.g.*, RFLP and SSR). In such cases, capillary electrophoresis can be used to analyze polymorphism. Such techniques are described in detail in U.S. Patent Nos. 5,534,123 and 5,728,282, which are incorporated herein by reference. Briefly, capillary electrophoresis tubes are filled with the separation matrix. The separation matrix contains hydroxyethyl cellulose, urea and optionally formamide. The RFLP or SSR samples are loaded onto the capillary tube and electrophoresed. Because of the small amount of sample and separation matrix required by capillary electrophoresis, the run times are very short. The molecular sizes and therefore the number of nucleotides present in the nucleic acid sample are determined by techniques described herein.

Electrophoresis can also be performed in microchannel plates. These plates have channels less than 100 μ in diameter etched on a solid substrate. By virtue of their smaller dimensions, they allow for even faster separations of nucleic acids in a separation matrix. Using these etched plates, samples can be evaluated with a high throughput format.

In another high throughput format, multiple capillary tubes are placed in a capillary electrophoresis apparatus. Samples are loaded onto the tubes and electrophoresis of the samples is run simultaneously. See, for example, Mathies & Huang, *Nature* 359:167 (1992). Because the separation matrix is of low viscosity, after each run, the capillary tubes can be emptied and reused.

The following examples are offered to further illustrate specific aspects of the present invention and are not to be interpreted so as to limit the scope of the present invention.

EXAMPLE 1

Effect of Genetic Matching on Sample Size and Confidence

The invention can be illustrated by the example of studying serum cholesterol and the effects drugs may have on this biological condition. It has been established that up to 80% of the variance in serum cholesterol can be attributed to genetics (see, for example, R. A. King, J. I. Rotter & A. G. Motulsky, THE GENETICS BASIS OF COMMON DISEASE, Oxford University Press, 1992).

The utility of matching patients at genetic loci is that the confidence, sample size or discriminating power of a given study can be favorably affected by genetic matching. For example, if a study is required to have 80% power to detect a difference of 20mg/dl at the 5% level and za =1.645 (representing the value from the standard normal distribution which is exceeded in 5% of cases) and zb = 0.842 (the value of the standard normal distribution that is exceeded in 80% of cases) the minimum sample size may be calculated as follows:

za <- qnorm (al) [.95 @ 1.645]

zb <- qnorm (be) [.8 @.842]

If the genetic contribution to the variance of a variable x is 80% and the variance is 1600 (the standard deviation squared for cholesterol) then the sample size is:

$$2 \times (za + zb)^2 \times \frac{\text{variance}}{(\text{difference})^2}$$

or

$$2 \times (2.49)^2 \times \frac{1600}{(20)^2} = \frac{[1600]}{[400]}$$

or

50

Thus 50 patients per arm of the study are needed to see a difference of cholesterol of 20mg/dl. If the patients are genetically matched (*i.e.*, the 80% contribution to variance is eliminated), the number of patients for each arm is reduced to 10.

[1600 x .80% = 1280, 1600-1280 = 320; 320 400 = .8]

The power of genetic matching can be realized in two other ways. For example, using the same number of patients (50) in each arm, with genetic matching the difference that can be resolved with the same power drops from 20 to 8.8. Likewise, the power of the study increases from .8 to greater than 0.99 assuming genetic matching and a difference of 20. If genetic matching cannot be fully achieved, some degree of matching can still have a favorable impact on such studies. This is illustrated in Table 1 below.

TABLE 1

Matching %	Variance Reduction	New Variance	n=Patients
10	128	1472	45
20	256	1344	41
30	384	1216	37
40	512	1088	33
50	640	960	29

EXAMPLE 2

Use of genetic analysis to reduce the sample size necessary in clinical trials

The following example is illustrative of the method of identifying underlying genetic factors that influence the response to treatment and the use of this information in the design of clinical trials.

A. Genetic Sub-Populations

In the instance of two distinct genetic sub-populations A and B, associated with a low response and high response to treatment, respectively, the response of treated individuals from the first sub-population, A, has mean μ_A and variance, σ_A^2. In the second sub-population, the mean and variance of response are given by μ_B and σ_B^2, respectively. No assumption is made about the shape of either distribution (i.e. they do not have to be normal). In control individuals who do not receive the treatment (instead receiving a placebo), the mean and variance of response is given by $(\hat{\mu}_A, \hat{\sigma}_A^2)$ and $(\hat{\mu}_B, \hat{\sigma}_B^2)$ for populations A and B, respectively. When a sample is taken from one of these populations, the distribution of the sample mean is normally distributed. For example, if a sample of size N of treated individuals is drawn from sub-population A, the sample mean has distribution $Norm[\mu_A, \sigma_A^2 / N]$.

If the genetic background of the sub-populations is ignored, both the treated (case) and untreated (control) populations include a mixture of individuals sampled from the two distributions. When the probability of an individual being chosen from genetic sub-population A is p and the probability of an individual being selected from genetic sub-population B is $q = 1 - p$, the distribution of the mean response of a sample selected from the two populations can be described.

For example, when N is the total population size and $\{x_1, x_2, ..., x_i, ...x_N)$

is a set of random variables, each describing an individual in the sample, the expectation of the mean response in the sample is given by,

$$E[\bar{x}] = \frac{1}{N}\sum_{i=1}^{N} E[x_i] \tag{1}$$

where $E[x_i] = \mu_A$ with probability p and $E[x_i] = \mu_B$ with probability $q = 1 - p$. So the mean of the distribution of the sample mean is,

$$E[\bar{x}] = p\mu_A + q\mu_B . \tag{2}$$

The variance of the mean response in such a case depends on both the variances of each of the two distributions (A and B) and on the difference between the means of these distributions. This variance can be expressed in terms of the sum of the variances of the individuals,

$$V[\bar{x}] = \frac{1}{N^2}\sum_{i=1}^{N} V[x_i] = \frac{V[x_i]}{N}. \tag{3}$$

When a random variable Y is defined such that when $Y = 0$, $V[x_i \mid Y = 0] = \sigma_A^2$ and when $Y = 1$, $V[x_i \mid Y = 1] = \sigma_B^2$, the expectation of this variance is then,

$$E[V[x_i \mid Y]] = p\sigma_A^2 + q\sigma_B^2. \tag{4}$$

Further, $E[x_i \mid Y=0]=\mu_A$ and $E[x_i \mid Y=1]=\mu_B$, hence,

$$V[E[x_i \mid Y]] = p\mu_A^2 + q\mu_B^2 - (p\mu_A + q\mu_B)^2 = pq(\mu_A - \mu_B)^2. \quad (5)$$

By standard theory, $V[x_i]=E[V[x_i \mid Y]]+V[E[x_i \mid Y]]$ so,

$$V[\bar{x}] = \frac{p\sigma_A^2 + q\sigma_B^2 + pq(\mu_A - \mu_B)^2}{N}. \quad (6)$$

Importantly, $V[\bar{x}] > Min[\sigma_A^2/N, \sigma_B^2/N]$. That is, the variance when the sub-populations are ignored is always larger than the variance of one of the sub-populations. This can intuitively be seen from the shape of equation 6. The variance is the weighted sum of the two population specific variances (σ_A^2, σ_B^2) plus a term representing the difference between the two population means $(\mu_A - \mu_B)^2$. Thus, the variance consists of both the within population variance and the between population variance.

The distribution of the sample's mean response (when the ratio of individuals from sub-populations A and B is $p:q$) is normal (by the Central Limit Theorem) and has mean and variance $(p\mu_A + q\mu_B, \frac{p\sigma_A^2 + q\sigma_B^2 + pq(\mu_A - \mu_B)^2}{N})$.

B. Marker Informativeness

The determination of which of the two genetic sub-populations (A and B) an individual belongs to can be made by examining genetic markers. Generally, the more markers that are genotyped, the greater the probability of assigning an individual to the correct group. In the absence of genetic information, a sample represents a random mix of the two populations with ratio $p:q$ of individuals from sub-populations A and B. If

the two genetic backgrounds have the same frequency, then $p = q = 0.5$ and the distribution of the sample mean is characterized as,

$$(\frac{\mu_A + \mu_B}{2}, \frac{\sigma_A^2 + \sigma_B^2 + 0.5(\mu_A - \mu_B)^2}{2N}). \quad (7)$$

In some instances, all individuals from the sub-population are genotyped for k equally informative markers. This is sometimes the case when markers are chosen at random (i.e. if nothing is known about genes involved in responsiveness). Additional markers will usually provide decreasing information (i.e. though the $k+1$ th marker increases the probability of correctly assigning an individual to a sub-population, it provides less information than the k th marker); this does not necessarily have to be the case but often is the case. For example, if there is *a priori* knowledge of the genes involved in response, these are typically examined first. Alternatively, if there is no information about the underlying genetics of response, then the genetic matching is based on relatedness (*i.e.*, the overall degree of genetic similarity in the genome) and hence the first few markers will be highly informative with diminishing information from each additional markers.

Consider a simple model where the probability of assigning an individual to the correct genetic sub-population when k markers have been genotyped is given by,

$$P[correct \mid k] = \frac{1}{2}(1 + \frac{k}{k+1}). \quad (8)$$

As k tends to infinity, the probability of correct assignment asymptotes to 1. This probability can be used in the equations above to determine the mixture of the sampled population. For $k \to \infty$, $p \to 1$ in equations 2 and 6, so the mean and variance of the sample mean is given by $(\mu_A, \sigma_A^2 / N)$ for population A and $(\mu_B, \sigma_B^2 / N)$ for population B (using the information to set $p = 0$ and so select non-responders) as expected.

C. Power of the clinical trial with genetic matching.

In a clinical trial that consists of a cohort of patients, half of whom are given the treatment and half who are given a placebo, wherein the two genetic sub-populations are equi-frequent, then the response in the treated sample is normally distributed with mean and variance given by,

$$(p\mu_A + q\mu_B, \frac{p\sigma_A^2 + q\sigma_B^2 + pq(\mu_A - \mu_B)^2}{N}). \quad (9)$$

In the control (placebo) sample, the distribution of the mean is again normal with mean and variance,

$$(p\hat{\mu}_A + q\hat{\mu}_B, \frac{p\hat{\sigma}_A^2 + q\hat{\sigma}_B^2 + pq(\hat{\mu}_A - \hat{\mu}_B)^2}{N}). \quad (10)$$

For the sake of simplicity, the two samples are selected to be of equal size, but this does not have to be so. When N is reasonably large (>30), standard theory of normal distributions can be used to show that the necessary sample size to detect a difference in response between the treated and placebo groups at the $\alpha\%$ level with power β is,

$$\frac{(Z_\alpha\sqrt{p\hat{\sigma}_A^2 + q\hat{\sigma}_B^2 + pq(\hat{\mu}_A - \hat{\mu}_B)^2} + Z_{1-\beta}\sqrt{p\sigma_A^2 + q\sigma_B^2 + pq(\mu_A - \mu_B)^2})^2}{(p(\hat{\mu}_A - \mu_A) + q(\hat{\mu}_B - \mu_B))^2} \quad (11)$$

where N is the number in each arm of the trial, giving $2N$ as the total number of individuals. From this equation it can be seen that the sample size increases as the difference between the means of the cases and controls decreases (*i.e.*, when the means

are identical $\hat{\mu}_A = \mu_A$ and $\hat{\mu}_B = \mu_B$, the sample size is infinity). The sample size also increases as the variances of the sub-populations increase.

D. Example of the sample size for matched and unmatched populations.

In one instance, the two genetic sub-populations have the same response characteristics when no treatment is administered ($\hat{\mu}_A = \hat{\mu}_B = 0$, $\hat{\sigma}_A = \hat{\sigma}_B = 8$) and the mean response to treatment of individuals from group A is described by $\mu_A = 5, \sigma_A = 8$. Response to treatment for individuals from group B is the same as for the placebo (*i.e.*, they are non-responders) with $\mu_B = 0, \sigma_B = 8$. Further, in this example, a 5% significance level ($\alpha = 0.05$) is used and the sample size represents the minimum number of individuals needed for 80% power ($\beta = 0.8$). Table 2 below gives the number of markers (k), the probability of the selected individual coming from group A (*i.e.*, being correctly identified as a responder) (p), the variance of the sample mean for the treated population ($V[\bar{x}]$) and the sample size required in each arm of the trial (N).

TABLE 2

k	p	$V[\bar{x}]$	N
0	0.50	70	83
1	0.75	69	36
5	0.92	66	24
10	0.95	65	22
∞	1.00	64	20

In this example, the within population variance is fixed at 64 for both genetic subpopulations and in both treated and untreated samples. Table 2 shows that, when no markers are genotyped, the variance is 70. This increase in the variance is entirely due to the difference in the response due to the underlying genotype. When this

is accounted for (when $p \to 1$), the variance returns to the expected value of 64. This inflation in variance has a marked effect on the necessary sample size (note in equation 11, the sample size does not increase linearly increasing variance, but rather with the square of the variance). Where 83 individuals are needed in each arm of the trial if no genetic information is available, only 20 individuals are needed if individuals can be correctly assigned as responders using their polymorphic profile.

EXAMPLE 3

Effects of linkage disequilibrium and marker allele frequencies on the power of a clinical trial

In this example, there are two genetic sub-populations A and B, and a single bi-allelic SNP (single nucleotide polymorphism) that is present in both sub-populations. One of the alleles, labeled g, has frequency $p[g \mid A] = p_A$ in sub-population A and $p[g \mid B] = p_B$ in sub-population B. In this particular example, the two sub-populations have equal frequency (i.e. $p(A) = p(B) = 0.5$). In this situation, the population-wide frequency of the rare allele (*i.e.*, the allele of the pair that has the lower frequency in the general population) is then, $p(g) = (p_A + p_B)/2$. In this example, it has been shown that the allele g is associated with increased response to the treatment. The increased response can be due to the function of the allele itself, but more usually arises due to the allele being in linkage disequilibrium with some (unknown) genetic factor or mutation. The magnitude of linkage disequilibrium, d, is defined as $d_A = p(A \,\&\, g) - p(A)p(g)$ for sub-population A and $d_B = p(B \,\&\, g) - p(B)p(g)$ for sub-population B. If the factor causing increased response is more common in sub-population A and the SNP marker is in close proximity, it is expected that $d_A > d_B$. In this sense, the sub-populations can be regarded as carrying the high-response (A) and low-response (B) alleles of the unknown gene that influences an individual's response to treatment.

In some instances, an SNP lies in a region that is known to harbor a gene

involved in response to treatment and sub-population A consists of high responders whereas sub-population B consists of low responders. In instances such as this, the SNP can then be used to determine which population an individual comes from and hence whether or not they are enrolled in a clinical trial. For example, when $p_A = .6$ and $p_B = .2$, this implies that individuals carrying the g allele are three times as likely to come from sub-population A and from sub-population B. As such, this marker is informative about whether an individual will respond well to the treatment (*i.e.*, whether they come from sub-population A). When the two sub-population are equi-frequent, then $p(A \& g) = 0.3$ and $p(B \& g) = 0.1$, hence

$$d_A = p(A \& g) - p(A)p(g) = 0.3 - 0.5 \times 0.4 = 0.1, \quad (1)$$

and

$$d_B = p(B \& g) - p(B)p(g) = 0.1 - 0.5 \times 0.4 = -0.1. \quad (2)$$

That is, there is a positive association between the g allele and an individual belonging to sub-population A. Conversely, there is negative association between g and sub-population B. With these measures of linkage disequilibrium, it is possible to calculate the conditional probability of an individual being a high responder (coming from sub-population A) given the individual carries the g allele. This is given as,

$$p[A \mid g] = \frac{d_A}{p(g)} + p(A) = 0.75, \quad (3)$$

and similarly,

$$p[B \mid g] = \frac{d_B}{p(g)} + p(B) = 0.25. \quad (4)$$

Note that by substituting for d_A and d_B, these conditional probabilities can also be expressed (using Bayes' theorem) as,

$$p[A \mid g] = \frac{p[A \& g]}{p[g]} = \frac{p_A}{p_A + p_B}, \quad (5)$$

and

$$p[B \mid g] = \frac{p[B \& g]}{p[g]} = \frac{p_B}{p_A + p_B} \quad (6)$$

That is, the strength of the association is encapsulated in the difference between the allele frequencies in the sub-populations.

Using information on this SNP increase, the probability of correctly choosing high responders from the null of 50% (given the two sub-populations are equally frequent) to 75%. This probability is a function of the frequency of the associated marker allele, the frequency of the allele influencing response and the strength of linkage disequilibrium between the two alleles. The necessary sample size can then be calculated as described in the previous section with $p = 0.5$ representing random sampling of individuals from the two populations and $p = 0.75$ representing sampling using the information from the genetic marker.

Note that in the example above, it is assumed that the allele frequencies in the two populations are known. This will often be the case if the polymorphism is already known to be involved in response but may not generally be known. However, it may often be the case that previous trials can be used to estimate allele frequencies in responders and non-responders.

This method extends naturally to multiple markers. In this case,

$G \in \{g_1,...,g_k\}$ represents the genotype of an individual at a set of bi-allelic markers with frequencies $\{p_1,...,p_k\}$ in sub-population A and $\{q_1,...,q_k\}$ in sub-population B. Then, by Bayes' Theorem,

$$p[A \mid G] = \frac{p[G \mid A]p[A]}{p[G \mid A]p[A] + p[G \mid B]p[B]} = \frac{\prod_{i=1}^{k} p_i}{\prod_{i=1}^{k} p_i + \prod_{i=1}^{k} q_i} \qquad (7)$$

where sub-populations A and B are equally frequent. Similarly, $p[B \mid G]$ can be expressed as,

$$p[B \mid G] = \frac{p[G \mid B]p[B]}{p[G \mid A]p[A] + p[G \mid B]p[B]} = \frac{\prod_{i=1}^{k} q_i}{\prod_{i=1}^{k} p_i + \prod_{i=1}^{k} q_i}. \qquad (8)$$

These values can then be used as in the single locus case to determine the probability of allocating an individual to the correct sub-population and hence to calculate the sample size.

For example, suppose five markers are genotyped and it is known that for all of them the rare allele has frequency 0.4 in sub-population A and 0.5 in sub-population B. The study is conducted with patients from sub-populations A and individuals from sub-population B, the latter individuals being known not to respond to treatment. Patients are enrolled dependent on the outcome of the 5 markers. Using equations 7 and 8, the probability of an individual belonging to sub-population A when the individual has the rare allele at n of the 5 markers is as shown in Table 3.

TABLE 3

Number of markers at which the rare allele is seen.	Probability of belonging to sub-population A
0	0.713
1	0.620
2	0.525
3	0.424
4	0.330
5	0.247

Table 3 shows that the probability of correctly assigning an individual to the correct sub-population increases rapidly with the number of markers genotyped. In this example, the markers are randomly chosen (not known to be associated with a specific gene) and the difference in the frequency of the rare allele between the two sub-populations is chosen to be 0.1.

Information of the type given in Table 3 can be used directly to categorize individuals into sub-populations or as a criterion for enrollment into a trial. For example, individuals having the rare allele at 1 or 0 of the 5 polymorphisms can be included in a clinical trial. This increases the probability of the trial containing individuals that will respond to the treatment (where it is assumed that sub-population A responds to treatment and sub-population B does not). In the case of individuals with no rare alleles, the probability of belonging to sub-population A is 0.713 and for individuals with 1 rare allele, the probability of belonging to sub-population A is 0.620. As such, this selection criterion greatly increases the proportion of individuals from sub-population A enrolled in the clinical trial. This simple method of selection is only illustrative and many other more complex procedures (for example, cluster analysis) can be employed depending on the number of polymorphisms and their respective allele frequencies.

EXAMPLE 4

Effects of the number of markers and their allele frequencies on the power of the polymorphic profile to discriminate between distinct groups of patients.

In this example, there are two types of markers, those with common alleles (*i.e.*, the two alleles are at similar frequency) and those with a rare allele. For the first marker-type, one allele has frequency $p_1 = 0.5$ in the sub-population A (responders) and $q_1 = 0.4$ in the sub-population B (non-responders). For the second type of markers, one allele has frequency $p_2 = 0.1$ in sub-population A and $q_2 = 0.08$ in sub-population B. In both cases, the rare allele has a 20% lower frequency in sub-population B compared to its frequency in sub-population A. A set of markers, K in number, are genotyped in a sample of 2000 patients who are known to belong either to sub-population A or sub-population B (from a previous clinical trial). For each of these individuals there are k observations $x_1, x_2, x_3, ..., x_k$ which take the value 0 if the rare allele is present and 1 otherwise. These individuals can be classified into sub-populations with $y = 0$ if they come from sub-population A and $y = 1$ if they belong to sub-population B. Using such training data, 2000 individuals were generated and assigned to one sub-population or the other using a linear logistic model (Christensen, *Log Linear Models and Logistic Regression*, Springer Verlag, New York, 1997) of the form,

$$\log[\frac{P(y=1)}{P(y=0)}] = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + ... + \beta_k x_k$$

Other statistical methods (such as described in section those in Example 3) can also be used. This linear logistic model was chosen to illustrate another method of classification.

Table 4 gives the probability of assigning an individual to the correct sub-population for 2, 5, 10, 20 and 50 markers. Values are given for both types of markers and for a mixture of the two. In this example, all markers are assumed to be independent of one another. If this were not the case, other, more powerful, statistical methods can be applied

(for example, methods of classification trees (Breiman *et al.*, Classification and Regression Trees, CRC Press, 1984).

TABLE 4

	Number of markers (k)						
	0	2	5	10	20	50	100
Common allele $p_1 = 0.5, q_1 = 0.4$	0.50	0.58	0.58	0.62	0.66	0.75	0.84
Rare allele $p_2 = 0.1, q_2 = 0.08$	0.50	0.50	0.53	0.55	0.56	0.57	0.62
Equal mix of $p_1 = 0.5, q_1 = 0.4$ and $p_2 = 0.1, q_2 = 0.08$	0.50	0.56	0.58	0.57	0.62	0.70	0.77

In these simulations, the markers for which the two alleles have similar frequencies are more effective in determining which group and individual belongs to, than are markers with very different allele frequencies. An equal mixture of markers from these two classes provides, as expected, intermediate results.

In many cases, data from previous clinical trials is available, but there is no information about which of the two sub-populations the responders and non-responders are drawn from. Such a scenario is more amenable to analysis by clustering methods. Data for 2000 individuals was simulated using the same allele frequencies as described above. These data were analyzed using K-means clustering (with K=2) to investigate how well the sub-populations can be defined by the markers alone. Because less information is available, there is little power in this method when very few markers ($k < 10$) are used. Results are shown in table 5 for 10 or more markers.

TABLE 5

	Number of markers (k)			
	10	20	50	100
Common allele $p_1 = 0.5, q_1 = 0.4$	0.58	0.52	0.69	0.77
Rare allele $p_2 = 0.1, q_2 = 0.08$	0.53	0.50	0.57	0.53
Equal mix of $p_1 = 0.5, q_1 = 0.4$ and $p_2 = 0.1, q_2 = 0.08$	0.52	0.52	0.68	0.75

In this example, markers for which both alleles are common perform better than those for which one allele is very rare. These results using cluster analysis correctly assign individuals with a lower probability than in Table 4. This is to be expected since less information was available *a priori* for these simulations.

As illustrated in part by the foregoing examples and the above description, the present invention has a number of uses with regard to treatment studies generally, and clinical trials in particular. For example, the invention includes the use of a polymorphic profile to conduct a clinical trial on a population of patients having the same disease, wherein the polymorphic profile includes at least one polymorphic site not known to be associated with the disease. The invention also includes the use of a polymorphic profile to conduct a reanalysis of data from a clinical trial in which statistical significance is determined on subpopulations of original treated and control groups selected for similarity of polymorphic profile. The invention also includes the use of a polymorphic profile to divide a population of individuals subject to a clinical trial into a plurality of subsets, the members of a subset showing greater similarity of polymorphic profile to each other than members in different subsets.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby expressly incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent or patent application were specifically and individually indicated to be so incorporated by reference.

WHAT IS CLAIMED IS:

1. A method for assessing a treatment procedure, comprising:

(a) selecting treated and control subpopulations of subjects from treated and control populations of subjects, the treated population being treated with a treatment procedure and the control population being treated with a control procedure; the subjects in both the treated and control populations having been characterized for polymorphic profile, and the subjects in both the treated and control subpopulations being selected for similarity of polymorphic profile;

(b) determining whether there is a statistically significant difference in a test parameter between the treated and control subpopulations as an assessment of the treatment procedure.

2. The method of claim 1, further comprising performing a further cycle of the selecting and determining steps on a second treated and control population.

3. The method of claim 1, wherein the treated and control subpopulations are selected for similarity to a first polymorphic profile, and the second treated and control subpopulations are selected for similarity to a second polymorphic profile.

4. The method of claim 1, wherein the treatment procedure comprises administering a pharmaceutical agent to the members of the treated population.

5. The method of claim 1, wherein the treatment procedure comprises administering a pharmaceutical agent to the members of the treated population and the control procedure lacks administration of the pharmaceutical agent to the members of the control population.

6. The method of claim 1 wherein the treatment procedure comprises administering a pharmaceutical agent to the members of the treated population and the control procedure comprises administering a placebo to the members of the control population.

7. The method of claim 1, wherein the treatment procedure comprises administering a first pharmaceutical agent to the members of the treated population and the control procedure comprises administering a second pharmaceutical agent that differs from the first pharmaceutical agent to the members of the control population.

8. The method of claim 7, wherein each of the first and second pharmaceutical agents is a combination of pharmaceutical agents.

9. The method of claim 1, wherein the treatment procedure comprises administering a first quantity of a pharmaceutical agent to the members of the treated population and the control procedure comprises administering a second quantity of the pharmaceutical agent that differs from the first quantity to the members of the control population.

10. The method of claim 1, wherein the treatment procedure comprises administering a pharmaceutical agent to the members of the treated population according to a first schedule and the control procedure comprises administering the pharmaceutical agent to the members of the control population according to a second schedule that differs from the first schedule.

11. The method of claim 1, wherein the treatment procedure comprises a behavioral therapy.

12. The method of claim 11, wherein the behavioral therapy comprises a diet regime.

13. The method of claim 11, wherein the behavioral therapy comprises an exercise regime.

14. The method of claim 1, wherein the test population comprises a plurality of plants and the treatment procedure comprises administering an agricultural agent to the plurality of plants, the agricultural agent selected from the group consisting of a herbicide, an insecticide and a growth-stimulating agent.

15. The method of claim 1, wherein the subpopulations are humans, animals or plants.

16. The method of claim 15, wherein the subpopulations are humans.

17. The method of claim 15, wherein the subpopulations are plants.

18. The method of claim 1, wherein the subpopulations are bacteria.

19. The method of claim 1, wherein the subpopulations of subjects are selected as having been similarly exposed to an environmental factor.

20. The method of claim 1, wherein the subpopulations of subjects are selected as having been differentially exposed to at least one environmental factor.

21. The method of claim 1, wherein the subpopulations of subjects are selected as being from the same ethnic group.

22. The method of claim 1, wherein the subpopulation of subjects are selected for common phenotypic trait.

23. The method of claim 1, wherein the subpopulations from the treatment and control populations each include at least 5 members.

24. The method of claim 23, wherein the subpopulations each include at least 10 members.

25. The method of claim 24, wherein the subpopulations each include at least 100 members.

26. The method of claim 1, wherein the polymorphic profile for each of the subpopulations is a single polymorphic form.

27. The method of claim 1, wherein the polymorphic profile for each of the subpopulations comprises a plurality of polymorphic forms.

28. The method of claim 27, wherein the polymorphic forms are present in the encoding regions of a plurality of genes.

29. The method of claim 28, wherein the plurality of genes encode enzymes in a metabolic pathway.

30. The method of claim 29, wherein the treatment procedure is a potential method for treating a disease and at least a subset of the plurality of genes is correlated with the disease.

31. The method of claim 29, wherein the treatment procedure is a potential method for treating a disease and the metabolic pathway is correlated with the disease.

32. The method of claim 1, wherein the polymorphic profile for each of the subpopulations includes at least 10 polymorphic forms.

33. The method of claim 32, wherein the polymorphic profile for each of the subpopulations includes at least 100 polymorphic forms.

34. The method of claim 1, wherein the polymorphic profiles for the subpopulations are at least 10% identical.

35. The method of claim 34, wherein the polymorphic profiles for the subpopulations are at least 50% identical.

36. The method of claim 35, wherein the polymorphic profiles for the subpopulations are at least 75% identical.

37. The method of claim 36, wherein the polymorphic profiles for the subpopulations are identical.

38. The method of claim 1, wherein the test parameter is a measure of a disease.

39. The method of claim 38, wherein the disease is cancer.

40. The method of claim 38, wherein the disease is correlated with an elevated serum cholesterol level.

41. The method of claim 1, wherein the test and control populations comprise plants and the test parameter is selected from the group consisting of a measure of susceptibility to herbicides, susceptibility to insecticides, susceptibility to a disease and susceptibility to frost damage.

42. A method for conducting a clinical trial, comprising:

(a) treating a treated population of patients having a disease with a drug and treating a control population of patients having the disease according to a control procedure;

(b) selecting a subpopulation of patients from each of the treated and control populations that have a similar polymorphic profile; and

(c) determining whether treatment with the drug correlates with status of the disease in the subpopulations as an assessment of the efficacy of the drug in treating the disease.

43. The method of claim 42, wherein the determining step comprises determining whether there is a statistically significant difference in a test parameter between the subpopulations.

44. The method of claim 42, further comprising determining a polymorphic profile for each patient in the treated and control populations before the selecting step.

45. The method of claim 42, wherein the control procedure involves administering a placebo to the members of the control population.

46. The method of claim 42, wherein the drug is effective in treating a disease other than the disease for which the clinical trial is being performed.

47. A method for assessing a treatment procedure, comprising:

(a) providing a database comprising

(i) designations for each member of a treated population treated according to a treatment procedure and for each member of a control population treated according to a control procedure;

(ii) designations for a polymorphic profile for each member of the treated and control populations; and

(iii) designations for a test parameter for each member of the treated and control populations;

(b) selecting a subpopulation from each of the treated and control populations for similarity in polymorphic profile; and

(c) determining whether there is a statistically significant difference in the test parameter between the subpopulations; and

(d) displaying an output of the result from the determining step.

48. A computer program product for assessing a treatment procedure, comprising:

(a) code for providing or receiving data comprising

(i) designations for each member of a treated population treated according to a treatment procedure and for each member of a control population treated according to a control procedure;

(ii) designations for a polymorphic profile for each member of the treated and control populations; and

(iii) designations for a test parameter for each member of the treated and control populations;

(b) code for selecting a subpopulation from each of the treated and control populations that have a similar polymorphic profile;

(c) code for determining whether there is a statistically significant difference in the test parameter between the subpopulations;

(d) code for displaying an output of the result from step (c); and

(e) a computer readable storage medium for holding the codes.

49. A system for assessing a treatment procedure, comprising:

(a) a memory;

(b) a system bus; and

(c) a processor operatively disposed to

(i) provide or receive data comprising

designations for each member of a treated population having been treated according to a treatment procedure and each member of a control population treated according to a control procedure;

designations for a polymorphic profile for each member of the treated and control populations; and

designations for a test parameter for each member of the treated and control populations;

(ii) select a subpopulation from each of the treated and control populations that have a similar polymorphic profile;

(iii) determine whether there is a statistically significant difference in the test parameter between the subpopulations; and

(iv) display an output of the result from step (iii).

50. A method of conducting a clinical trial, comprising

(a) determining a polymorphic profile for individuals in a population having the same disease, wherein the polymorphic profile includes at least one polymorphic form at a polymorphic site not known to be associated with the disease;

(b) selecting a subpopulation of individuals having a similar polymorphic profile from the population;

(c) administrating a treatment regime to a treatment group within the subpopulation and a control regime to a control group within the subpopulation;

(d) determining a test parameter in patients in the treatment group and the control group expected to vary in response to an effective treatment regime; and

(e) determining whether the parameter shows a statistically significant difference between the treatment group and the control group.

51. A method of conducting a clinical trial, comprising:

(a) determining a polymorphic profile for individuals in a population having the same disease;

(b) identifying subsets of individuals in the population such that the individuals in a subset show greater similarity in polymorphic profile than individuals in different subsets;

(c) allocating the members of each subset to treatment and control subpopulations of the populations so that the treated and control subpopulations each receive at least one individual from each subset;

(d) administrating a treatment regime to the treatment subpopulation and a control regime to a control subpopulation;

(e) determining a parameter in patients in the treatment subpopulation and the control subpopulation expected to vary in response to an effective treatment regime; and

(f) determining whether the parameter shows a statistically significant difference between the treatment subpopulation and the control subpopulation.

52. The method of claim 51, wherein the subsets are pairs of individuals.

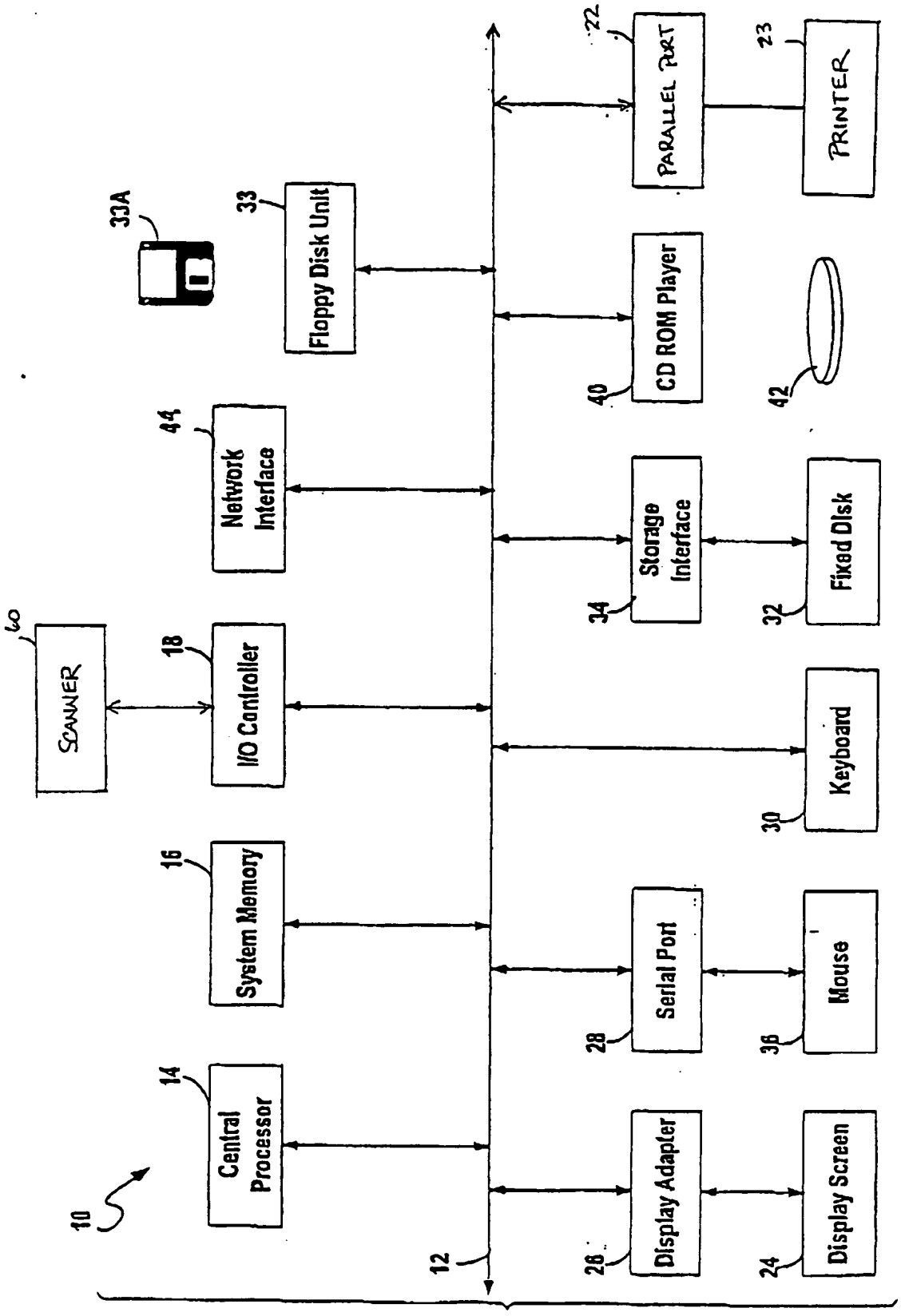
10
12
Central Processor
14
System Memory
16
I/O Controller
18
Network Interface
44
Floppy Disk Unit
33
33A
Display Adapter
26
Display Screen
24
Serial Port
28
Mouse
36
Keyboard
30
Storage Interface
34
Fixed Disk
32
CD ROM Player
40
42
PARALLEL PORT
22
PRINTER
23
SCANNER
60

FIG. 1

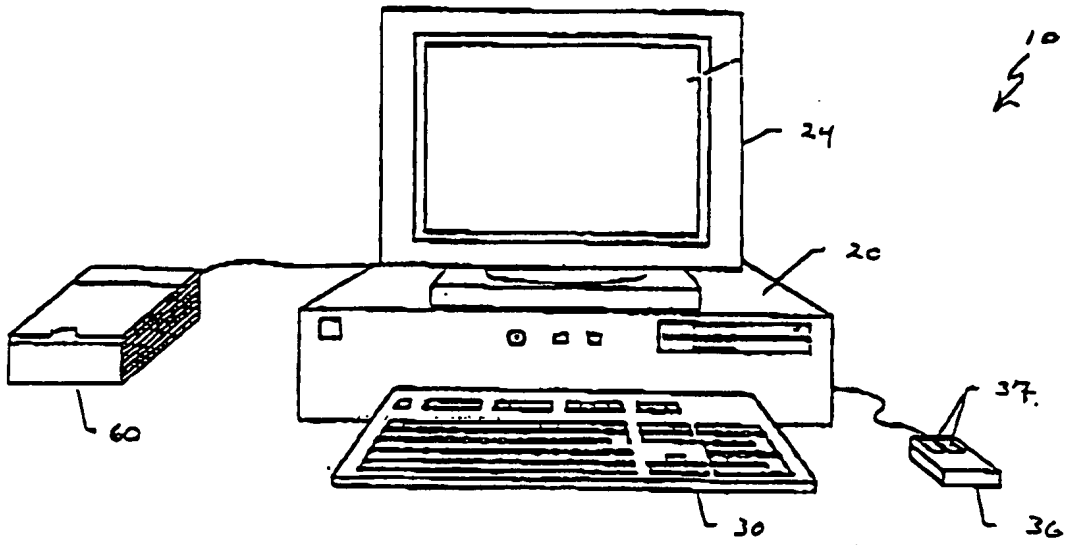

FIG. 2

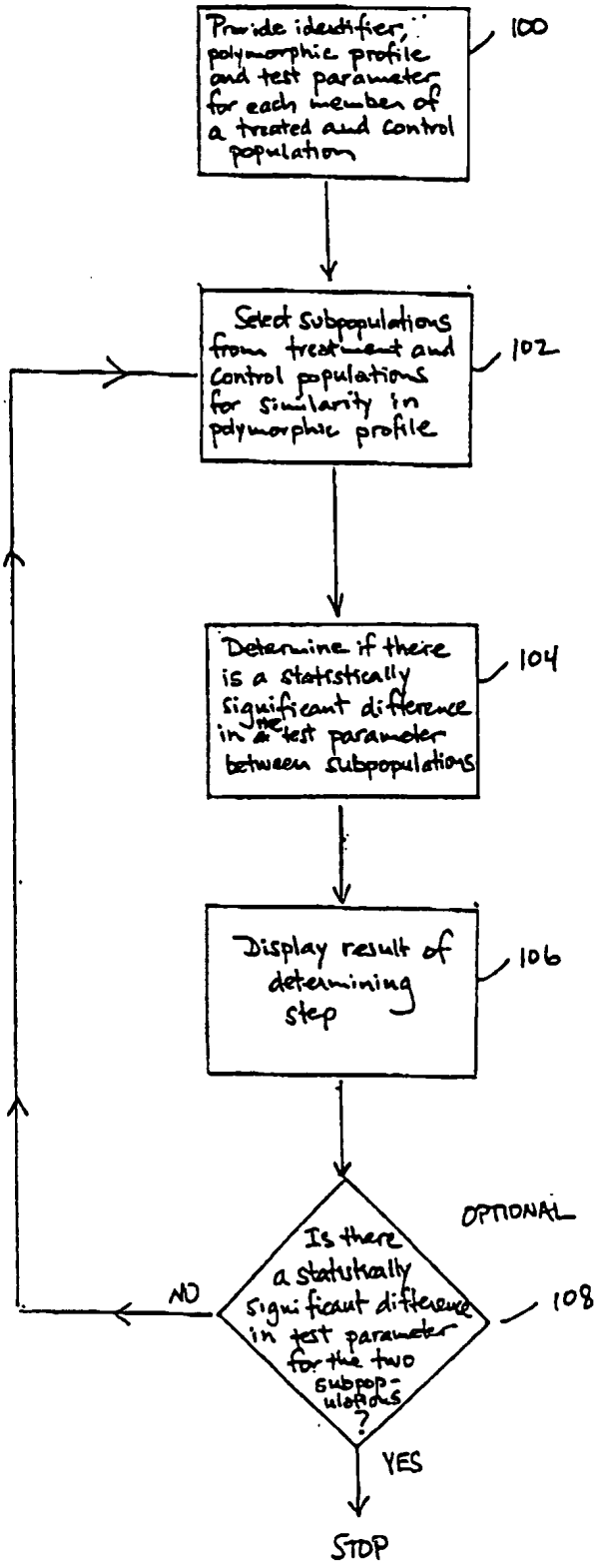
Provide identifier, polymorphic profile and test parameter for each member of a treated and control population
100
Select subpopulations from treatment and control populations for similarity in polymorphic profile
102
Determine if there is a statistically significant difference in the test parameter between subpopulations
104
Display result of determining step
106
OPTIONAL
Is there a statistically significant difference in test parameter for the two subpopulations ?
108
NO
YES
STOP

FIG. 3